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OSSEOUS METASTASIS OF CARCINOMA OF THE PROSTATE

WITH SPECIAL REFERENCE TO THE PERINEURAL LYMPHATICS

SHIELDS WARREN, M.D.

PAUL N. HARRIS, M.D.

AND

ROGER C. GRAVES, M.D.

BOSTON

The high frequency of metastasis of carcinoma of the prostate to the skeleton has long been recognized, ranging from over 70 per cent (Kaufmann [1]) to 30 per cent (Bumpus [2]). The frequency of involvement of the pelvis and the lumbar vertebrae has been noted by practically all observers, particularly Kaufmann,[1] Pürckhauer [3] and Copeland.[4] The origin of skeletal metastases from blood-borne emboli in accordance with the hemic hypothesis of von Recklinghausen [5] has been frequently accepted. However, it is difficult on this basis alone to explain the predilection for the pelvis and the vertebrae so frequently noted in prostatic cancer.

In metastasis of carcinoma of the breast, Handley [6] insisted on the importance of extension of the tumor into the bone from cancerous permeation of the adjacent deep fascial lymphatic plexuses. Willis,[7] with much justice, criticized certain of Handley's hypotheses regarding the origin of osseous secondary deposits of cancer of the breast. With many

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From the Pondville State Hospital for Cancer, the Laboratory of Pathology of the New England Deaconess Hospital and the Department of Pathology of Harvard University Medical School.

1. Kaufmann, E.: Deutsche Ztschr. f. Chir. **53**:381, 1902.
2. Bumpus, H. C.: Surg., Gynec. & Obst. **32**:31, 1921.
3. Pürckhauer, R.: Ztschr. f. Krebsforsch. **28**:68, 1929.
4. Copeland, M. M.: Arch. Surg. **23**:581, 1931.
5. von Recklinghausen, F. D.: Virchows Arch. f. path. Anat. **100**:503, 1885.
6. Handley, W. F.: Cancer of the Breast and Its Treatment, New York, Paul B. Hoeber, Inc., 1922.
7. Willis, R. A.: The Spread of Tumors of the Human Body, London, J. & A. Churchill, Ltd., 1934, p. 337.

of Willis' statements we are in accord. He stressed that if Handley's conception is to hold, namely, that bones suffer infestation as a sequel to continuous permeation of the fascial lymphatics, it must also imply regular infestation of the regional nodes. Kolodny [8] surmised that in metastasis of mammary cancer and possibly in that of prostatic cancer the order of frequency of involvement of various bones may denote centrifugal spread from the primary growth.

Roberts [9] in his study of the spinal metastases of prostatic cancer found that very frequently there are extensive growths and masses in the deep surfaces of the spinal laminae and of their ligaments, and he conceived that the lymphatics of these ligaments may be important means of extension of the tumor from one part of the axial skeleton to another. He regarded the sacral lymphatics as the channels by which the tumor reaches the vertebral column. The tumors which he studied were advanced, and the objection has been raised that possibly the neoplastic infiltration of the ligaments may have been secondary to deposits in the adjacent bone.

We believe, in view of the strikingly high frequency of osseous metastases in bones adjacent to the prostate when carcinoma of this gland exists, that some other mechanism than fortuitous hematogenous metastasis must come into play. It is quite obvious that many of the metastases in bone are hematogenous. Definite proof of this has been submitted so many times that further evidence is unnecessary. However, there must be some further factor to account for the peculiar frequency of involvement of the adjacent bones, particularly when the metastases occur in bones such as those of the pelvis, which are not involved with peculiar frequency in osseous metastases from mammary or pulmonary carcinoma. Of eighty-one cases of carcinoma of the prostate studied by Graves and Militzer,[10] 85 per cent showed roentgen evidence of metastasis to the pelvis and sacrum, 59 per cent to the lumbar spine and only 35 per cent to the femora and 23 per cent to the dorsal spine.

So far as the metastases in the central areas of bone are concerned there can be little doubt that the blood stream is the sole means of their dissemination. However, many of the metastases of prostatic cancer appear close to the cortex, and in some of the cases which we shall present later infiltration through the cortical ostia may be seen.

In our observations on autopsy and operative material and in clinical study of the patients, we were struck by the frequency of involvement of perineural lymphatics and also by the frequency with which pain, implying involvement of nerves, appeared as a relatively early symptom.

8. Kolodny, A.: Arch. Surg. **11**:690, 1925.
9. Roberts, O. W.: Brit. J. Surg. **15**:652, 1927.
10. Graves, R. C., and Militzer, R. E.: J. Urol. **33**:235, 1935.

Thus with a small, nonobstructing cancer of the prostate pain on micturition may be marked, whereas with a large, hypertrophied prostate micturition may be extremely difficult but not painful.

Studies of the soft tissue in the neighborhood of the prostate convinced us that the perineural lymphatics might be an important pathway of dissemination of the tumor. So far as we have been able to determine, while there have been numerous studies of the lymphatic drainage of the prostate, there has been no consideration of the perineural lymphatics as channels of tumor dissemination. In fact, Roberts stated casually, "The pelvic nerves appear free from invasion." Moore [11] called attention to the frequency of invasion of perineural lymphatics even in early stages of the disease.

The present study represents an attempt to evaluate the importance of this mode of spread of carcinoma of the prostate. It also affords an explanation of the peculiar frequency of pelvic and lower vertebral involvement in this disease.

MATERIALS AND METHODS

Cases of carcinoma of the prostate in which lower vertebral and pelvic metastases were known to exist and cases in which such metastases were not demonstrated during life were chosen. The autopsy material was derived from the Pondville State Hospital for Cancer. In all cases skeletal roentgenograms had been made, and all the patients had been under observation for periods of from few to many months.

The method of investigation was as follows: Instead of the routine procedure at autopsy, a block dissection was carried out to include the prostate, bladder, rectum and soft tissues of the pelvis, together with such of the adjacent pelvic bones as could be readily obtained, as well as the sacrum and the bodies of the lower lumbar vertebrae. The body was then satisfactorily restored. These masses of tissue, obtained by Dr. T. B. Eberhard, resident pathologist, were then fixed in solution of formaldehyde until sufficiently hardened to be handled without fear of distorting the tissue relationships. Horizontal sections were made through the tissue at intervals of approximately 5 mm. These sections were dehydrated, cleared, embedded in pyroxylin (celloidin) and sectioned on a large MacCallum microtome. The sections were stained by the alum-hematoxylin-eosin method and mounted in balsam. In instances in which the resulting sections could not be mounted on a slide 3¼ by 4¼ inches (8 by 10.8 cm.) they were divided into two or three portions in such manner that they could be readily reconstructed. In specimens containing bone, decalcification with 5 per cent nitric acid was carried out before the preliminary sectioning. Decalcification was extraordinarily difficult in some of the specimens with marked osteosclerotic metastases; not only was a long period of decalcification required, but the final sections were not entirely satisfactory. Relatively large masses of the bone were decalcified in order to avoid the distortion of tissue relationships that might result from sawing.

The resulting sections were sufficiently good to permit study under moderate power of the microscope. The major details could be determined fairly well by using the mounted slide as a lantern slide and projecting it against a screen for study.

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11. Moore, R. A.: J. Urol. **33**:224, 1935.

These sections have been supplemented by other sections obtained in a very considerable number of cases of carcinoma of the prostate from specimens removed surgically or at autopsy.

As will be seen in our cases, the cancer was usually advanced, although in one (case 8) it was unrecognized clinically. One case of carcinoma of the bladder was included as a control (case 6).

REPORT OF CASES

CASE 1.—An American farmer, aged 76, admitted Feb. 9, 1933, suffered from urinary incontinence which had begun following a suprapubic operation five weeks before. He had experienced pain in the left thigh for one year, rectal incontinence for eight months and loss of weight for two or three years. Examination disclosed a poor general condition, Parkinson's disease and a hard irregular mass filling the whole rectal cavity, almost occluding it.

Roentgenologist's Reports.—"February 13: The pelvis shows rounded areas of increased density varying from 1 to 3 cm. in diameter, scattered throughout. There is dense bone over the upper part of the sacrum. The second lumbar vertebra is also involved. The process is present in the left femur, from the greater trochanter down to the midshaft. The lower part of the femur is not demonstrated. The appearances are quite characteristic of metastatic disease from carcinoma of the prostate. In the kidneys, following intravenous injection of dye, the pelvis and calices are fairly well demonstrated. There is considerable tortuosity of the right ureter with slight dilatation."

"February 18: A portable examination of the chest shows an area of dulness in the upper right lung field which looks like a pneumonic process. I do not believe it is metastatic. There are a number of areas of density in the ribs, which are apparently metastases."

Biopsy.—Tissue removed from the rectum showed carcinoma; the material was insufficient for classification.

Treatment and Course.—A colostomy was done March 6. The patient died September 19. The interval between the onset of symptoms and the discovery of metastases was one year. The length of life after the discovery of metastases was seven months.

Autopsy.—The final diagnosis was: carcinoma simplex of the prostate with extension to the soft tissues of the pelvis and rectum and metastasis to the pelvis, spine and para-aortic lymph nodes; bronchopneumonia; mitral and aortic valvulitis with insufficiency; pyelonephritis; retention cysts of the kidneys.

The patient showed marked emaciation with a permanent colostomy opening in the anterior abdominal wall. The lower lobes of both lungs were dark red-purple, and a large amount of bloody fluid could be expressed, together with some yellow purulent material. The gastro-intestinal tract was normal except for the lower end of the rectum, which was closely adherent to the prostate and surrounding soft tissue and completely surrounded by tumor. The lumen was almost completely occluded by encroachment of the tumor. The involvement extended along the axis of the rectum about 5 cm. Both kidneys were essentially normal; the right weighed 140 Gm. and the left 160 Gm., and each had several thin smooth-walled cysts 1.5 cm. in diameter. The cortex was 3 mm. thick, dark red and poorly demarcated. In each kidney were several yellowish-red soft pyramidal foci with the bases toward the periphery. The pelvic mucosa showed slight injection and was thickened. The bladder was small and thick-walled; the mucosa,

smooth but injected. The trigon was elevated by a nodular bulbous fold extending transversally across it for 2 cm. and extending 0.4 cm. above the floor. On section, the floor of the bladder was continuous with the prostatic mass. The prostate was removed together with the bladder, the soft tissues of the pelvis, the coccyx, a portion of the sacrum and the lower lumbar vertebrae. The mass replacing the prostate was large, hard, yellowish white and granular, shading off into the surrounding tissue. The pelvic, iliac and para-aortic lymph nodes were enlarged up to 1.5 cm. in diameter, hard, somewhat adherent to surrounding structures and on section yellowish white and granular, with some foci of necrosis. The aorta showed marked atheromatous change. The body of the second lumbar and a part of the third lumbar vertebra were made up of yellowish-white eburnated bone. The remainder of the lumbar vertebrae, the sacral vertebrae and the coccyx were uninvolved in spite of the roentgen evidence of metastases in them.

Microscopic Observations: The tumor was made up of strands and clusters of closely packed small polyhedral cells of epithelial type. Occasionally these formed ill-defined acini. The cytoplasm was finely granular, rarely vacuolated and rather darkly stained. The nuclei were fairly large and hyperchromatic. Sections of the pelvic bones showed the marrow spaces heavily infiltrated with neoplastic growth. Sections of other bones revealed no evidence of the tumor.

In large sections the prostate was seen to be diffusely invaded by the tumor, and in the capsule the perineural invasion was striking and constant. The posterior wall of the bladder showed much invasion, but the ureters none. The muscularis of the rectum contained small masses of the tumor, and there was much infiltration of the tissues between the rectum, bladder and prostate. The tumor extended posterolateral to the bladder and up beside the rectum. The seminal vesicles were surrounded by the growth and showed much invasion. The muscularis of the terminal portions of the vasa deferentia showed some encroachment. In this region perineural invasion was seen very often but not so constantly as in the prostatic capsule. A small amount of the tumor was seen in the vicinity of the iliac vessels, some of which was perineural. Several tumor-invaded nodes were seen here and anterior to the lower vertebrae. The sacrum and lumbar vertebrae contained no growth, but there were foci up to 1 cm. in diameter in which the marrow was fatty and the trabeculae thin and less numerous than elsewhere. The periosteum contained no tumor.

Case 2.—An American artist, aged 72, admitted May 3, 1933, had had urinary frequency and urgency for nine months and complete retention for five weeks. There was pain in the right thigh. Roentgen therapy had been given. He had lost 29 pounds (13.2 Kg.) in one year. He was in poor general condition, the prostate was extremely large, uneven and very hard, and there was induration in the region of the seminal vesicles, especially on the left side.

Roentgenologist's Reports.—"May 5: Extensive hyperplastic metastatic malignant growth involves the sacrum, the right ilium and ischium and the left ischium. The metastases are most probably from a cancer of the prostate."

"June 2: The colon shows some enlargement throughout. The rectum is not as large as one would expect but the contour is regular. I cannot find any evidence of disease in the colon."

"July 20: The dorsal spine shows involvement of one of the lower dorsal vertebrae by metastatis from the prostate. The skull is uninvolved. The chest shows no evidence of metastasis."

Course.—Nausea and vomiting developed, with marked elevation of the nonprotein nitrogen in the blood. The patient died September 21. The interval

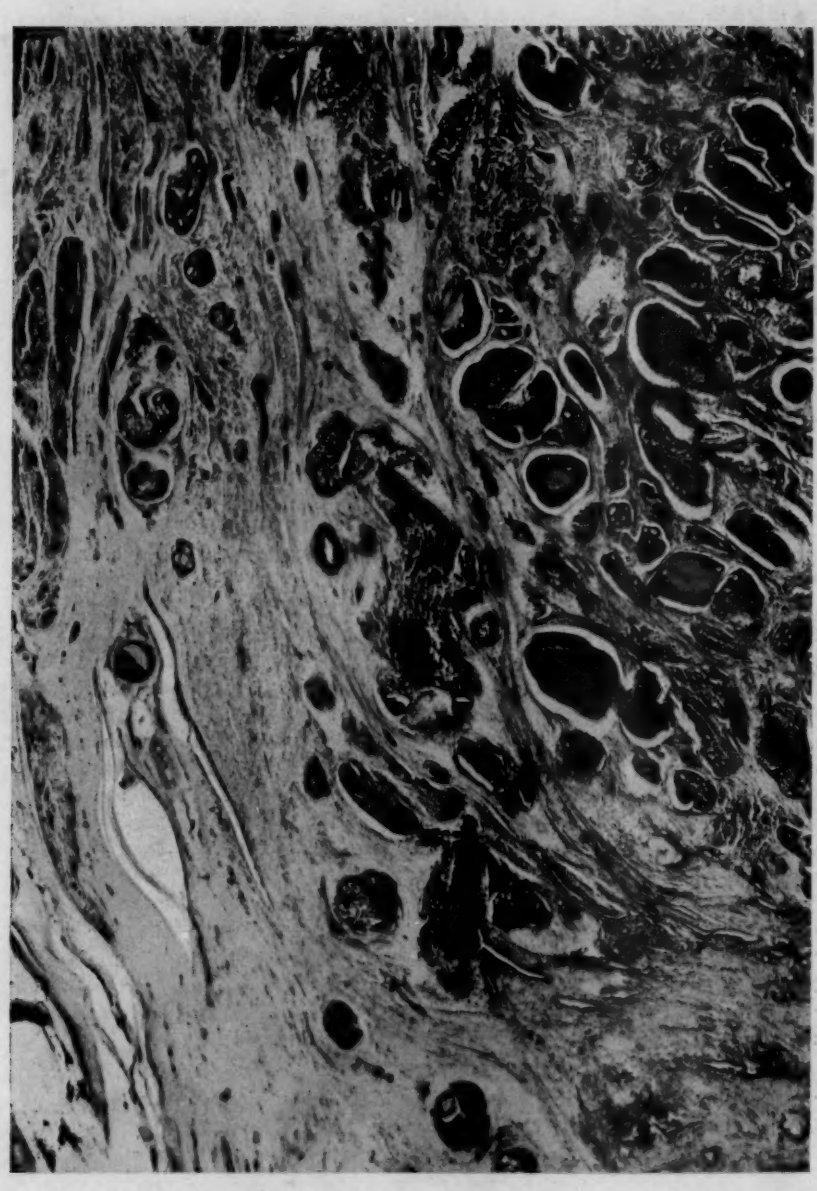

Fig. 1.—A photomicrograph of one of the large sections used in our study; × 18. The prostate shows widespread invasion of the tumor. Some lymphatics in the capsule are invaded, and in the left center is a large mass of the tumor in a perineural lymphatic.

between the onset of symptoms and the discovery of metastases was nine months or less. The length of life after the discovery of the metastases was less than one year.

Autopsy.—The final diagnosis was carcinoma simplex of the prostate with extension to the bladder and ureter; metastasis to the left and the right ilium, sacrum, second and third lumbar vertebrae and iliac, pelvic and para-aortic lymph nodes; pyelonephritis and pyo-ureter on the right; hydronephrosis and hydro-ureter on the left.

The body showed marked emaciation. The gross findings were essentially unimportant except for those in the genito-urinary system and bones. The right kidney weighed 120 Gm.; the left 125 Gm. The right kidney on section proved to be only a shell of renal substance, approximately 3 mm. in thickness, multilocular and filled with pus. The mucosa was hemorrhagic and covered with reddish-green slough. The left kidney showed a slightly dilated pelvis containing 20 cc. of clear urine; its cortex was 3 mm. in thickness and poorly demarcated. The right ureter was dilated to 1.5 cm., with the wall thick and inelastic and the lumen filled with pus. The left ureter was 2 cm. in diameter, thin-walled, containing clear urine. The bladder was empty, the wall contracted. The mucosa was thick, trabeculated and covered with yellowish-green slough. There was a soft reddish-gray spongy tumor, about 1.5 cm. in diameter, extending 0.5 cm. above the floor between the urethral orifice and the left ureteral orifice. The prostate was entirely replaced by yellowish-white granular hard tumor tissue which invaded the bladder and adjacent soft parts of the pelvis for about 1 cm. The seminal vesicles were lost in the mass of tumor. The pelvic, iliac and para-aortic lymph nodes were enlarged, hard and fixed to the surrounding tissue. The second and third lumbar vertebrae, all the sacrum and many foci in the ilium were composed of pale yellow eburnated bone infiltrated by the tumor.

Microscopic Observations: The tumor was made up of cords, masses and poorly formed acini of large irregular cells with finely granular cytoplasm and large vesicular nuclei. Mitoses were rare. The stroma was scanty. There was marked lymphatic invasion. The centers of some of the larger masses were necrotic. The wall of the bladder showed heavy neoplastic infiltration. Almost all the lymphoid tissue in the nodes was replaced by the tumor. Sections of vertebrae showed heavy infiltration.

The large sections showed moderate invasion of lymphatics in the muscularis of the rectum, and many of these were perineural. The mucosa and submucosa showed subacute inflammation. The prostate showed widespread invasion, and in the capsule was extensive and fairly constant perineural invasion. The bladder inferiorly, posteriorly and on one side contained moderate infiltration. The ureters contained no growth. There were small masses of the tumor between the prostate, the bladder and the rectum, and a few small masses were lateral to the rectum, much more on the left. One seminal vesicle was partly obliterated. The vesicles showed slight peripheral invasion, and they and the vasa were surrounded by tumor. A few lymphatics in the adventitia of arteries near the rectum contained the tumor. Some perineural invasion was present at a distance from the prostate, and beside the iliac vessels were large masses of the tumor in perineural lymphatics. One lymph node near the sacrum and two nodes near the vertebrae showed much invasion. Other nodes were normal.

The tumor was present in the sacrum and vertebrae and had caused much proliferation of bone. The periosteum contained small amounts of the growth. In a few foci extension into the bone appeared to be by way of penetrating lymphatics in cortical foramina. Elsewhere it appeared to be by direct extension.

Case 3.—An American mechanic, aged 56, admitted June 20, 1933, had had nocturia (four to five times) for one year and pain in the back for six months. High voltage roentgen treatment had been given anteriorly and posteriorly over the pelvis before admission. He had lost 15 pounds (6.8 Kg.) or more in the

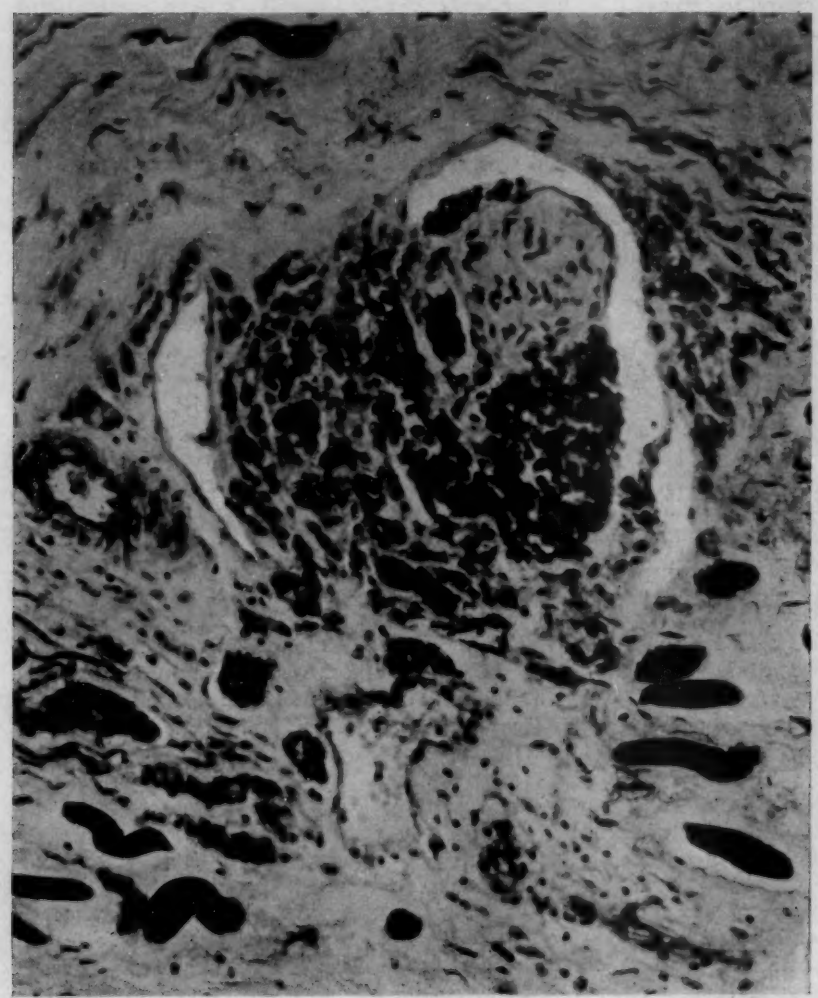

Fig. 2.—A prostatic capsule showing much distention of perineural lymphatics by tumor cells; × 235. Small masses of the tumor are present in other lymphatics. The coarse black structures are skeletal muscle fibers.

past year. He was in fair general condition. The prostate was very slightly enlarged and hard, with extension of the induration into the region of the right seminal vesicle.

Roentgenologist's Report.—"June 26: The pelvis shows no very definite evidence of metastatic disease. In the left pubis there is a slight irregularity in the appearance of the bone which may be due to a metastatic process. Very definite lesions are present in the dorsal spine. The ninth dorsal vertebra is beginning to narrow

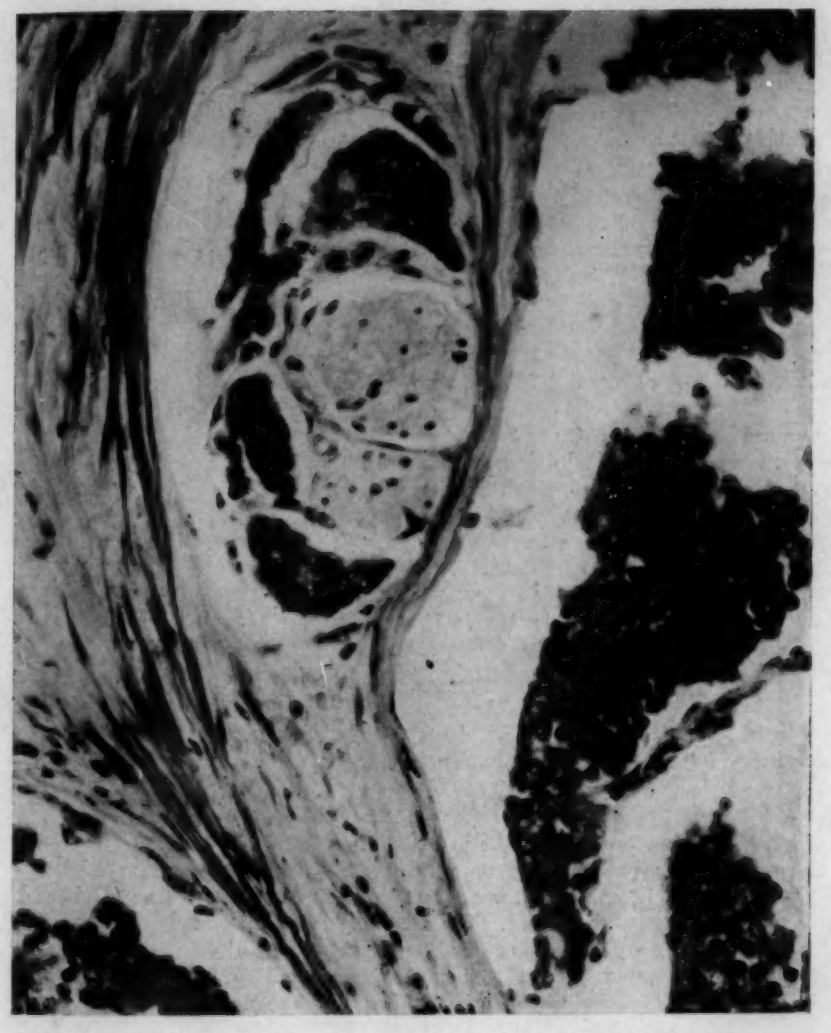

Fig. 3.—A section through the edge of the prostate showing several masses of the tumor in perineural lymphatics; × 560. The lymphatic walls are readily distinguished.

down. There is definite narrowing of the seventh with beginning wedging of the fifth. There is also pathologic fracture of the left clavicle with a beginning moth-eaten appearance around the margins of the fracture."

Treatment and Course.—High voltage roentgen therapy was given over the dorsal spine and the left clavicle. August 5, chordotomy was done for relief of pain. Pain persisted in the region of the left shoulder. The patient died September 27. The interval between the onset of symptoms and the discovery of metastases was about 10 months. The length of life after the discovery of metastases was from five to six months.

Autopsy.—The final diagnosis was: carcinoma simplex of the prostate with metastasis to the lungs, diaphragm, pleura, thyroid, peritoneum, kidney, liver, adrenals, spleen, eighth, ninth and eleventh dorsal and third and fifth lumbar vertebrae, left clavicle (pathologic fracture), sixth rib on the right, and pelvic, para-aortic, mesenteric and mediastinal lymph nodes; arteriolar nephrosclerosis; bilateral hydrothorax.

The body was that of a well developed and well nourished elderly white man. There were swelling and abnormal mobility of the outer third of the left clavicle. Significant were numerous nodules of yellow-white granular hard material over the peritoneal cavity and pleural cavities. Scattered small hard nodules were present throughout both lungs. The lungs were somewhat soggy. The gastrointestinal tract was normal except the last few centimeters of the rectum; there the muscularis and perirectal tissues were hard and apparently infiltrated by the tumor. The liver weighed 1,700 Gm. and showed evidence of central necrosis. The left adrenal showed nodules of gray tissue in the medulla. The kidneys weighed 170 Gm. each. On section the cortex was poorly demarcated, the glomeruli were prominent, and the pelvic mucosa showed injection. The bladder was empty. Its mucosa was thickened, showed injection and was covered with yellowish-green slough. The pelvic, para-aortic, mesenteric and mediastinal lymph nodes were enlarged, hard and adherent to the surrounding tissue. On section they appeared to be infiltrated with the tumor. The thyroid showed two metastatic nodules. The outer third of the clavicle showed a nodule, approximately 5 by 3 cm., which had completely destroyed the bone. The right sixth rib was involved. Nodules of the tumor were seen in the eighth, ninth and eleventh dorsal and the third and fifth lumbar vertebrae.

Microscopic Observations.—The microscopic observations essentially confirmed the gross findings with the addition that there was metastasis to the splenic pulp, invasion of the appendix by extension from the mesentery, focal invasion of the liver and metastasis to one kidney. The prostate was heavily infiltrated, showing large foci of necrosis with abundant stroma and considerable fibrosis.

The large sections showed the prostate diffusely invaded while in the capsule perineural and even endoneural invasion was striking and fairly constant. The bladder showed little invasion, but a cord of the tumor extended up a lymphatic in the serosal fat to the fundus. The ureters and vasa showed no invasion. The lower parts of the vesicles were filled by the tumor, but there was little in their walls. The tumor was present in the anal submucosa and sphincter, and here there was striking and fairly constant perineural invasion. The rectum was closely applied to the lower part of the prostate, and there was extensive invasion of its muscularis. Some invasion here was perineural. One nodule invaded the sigmoid from the serosa. There was little of the tumor between the rectum and the prostate, but many small nodules were seen in the peritoneum of the pouch of Douglas. A few small masses were seen lateral to the rectum, some perineural.

The coccyx contained no tumor. The sacrum and the lower vertebrae showed fairly extensive invasion with little destruction of bone. The upper vertebrae had none of the tumor. The periosteum contained moderate amounts with direct inva-

sion of bone in several places. Some of the tumor was present in perineural and perivascular lymphatics in the periosteum. The tumor could not be traced in an unbroken chain from the prostate to the bone, and embolic spread via perineural lymphatics seemed probable.

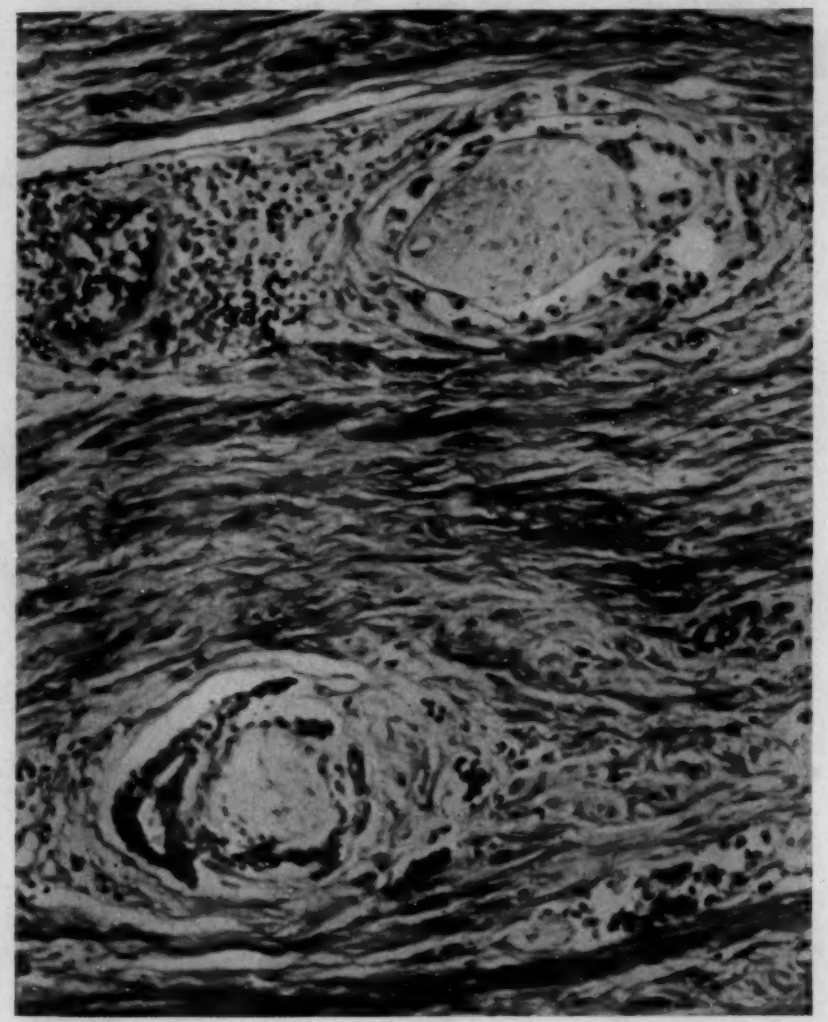

Fig. 4.—A section of the prostatic capsule; × 235. Invasion of the lymphatics about two nerves is seen. In one, the tumor cells form glands.

Case 4.—An American farmer, aged 88, admitted April 29, 1933, had had basal cell carcinoma of the left inner canthus for one year. There was a history of increasing constipation in the past year and of nocturia and partial incontinence, beginning with a sudden attack of complete retention three years before. There had

been pain in the lower part of the back and in the left knee for one year. No loss of weight was noted. The patient was in fair general condition. The nonprotein nitrogen of the blood was elevated. A large mass was seen in the region of the prostate, surrounding the rectum bilaterally and extending into the region of the seminal vesicles. No rectal ulceration was found by proctoscopy.

Roentgenologist's Reports.—"May 1: The pelvis and lumbar spine and the knee show extensive hypertrophic changes but no evidence of metastatic disease."

"June 19: The lung fields are essentially clear. There is probably slight dulness at the left base. The diaphragm is not seen on that side. This may be due to consolidation or a slight amount of fluid at the left base."

Treatment and Course.—Radium was applied to the lesions of the left inner canthus; suprapubic cystostomy was done. The patient died September 14, after a long decline marked by clinical signs of an infection of the urinary tract and lungs. The interval between the onset of symptoms and the discovery of metastases at autopsy was three years and five months.

Autopsy.—The final diagnosis was: adenocarcinoma of the prostate with metastasis to pelvic and para-aortic lymph nodes; basal cell carcinoma of the left inner canthus; bronchopneumonia; chronic nephritis; multiple colloid adenomatous goiter; adhesive pleuritis and hydrothorax; chronic mitral valvulitis; hypertrophy of the heart.

[Comment: This case and the preceding one offer a striking contrast between the small hard carcinomatous prostate with widely metastasizing growth in a relatively young man and the large prostatic mass with but few secondary deposits in an old man. Such differences in behavior have been commented on by Ferguson [12] and by Graves and Militzer.[10]]

The body was that of a well developed but markedly emaciated elderly white man. In the midline was a suprapubic cystostomy opening. The observations were essentially unimportant except for the following: There was soggy firmness of the lower lobes of both lungs with a large amount of yellow pus in the bronchioles. The gastro-intestinal tract was normal except for marked thickening of the submucosa and injection in the mucosa in the region of the prostate. Each kidney weighed 125 Gm. The small firm capsule of each kidney stripped with some difficulty from a reddish-brown raw granular surface. On section the cortex was 0.5 cm. in thickness and poorly demarcated. The pelves of the kidneys and the ureters were uninvolved. The bladder was empty, with trabeculation and injection in the mucosa and a thin layer of yellowish-green fibrin present. The floor of the bladder was elevated but not eroded. The prostate was enlarged to about twice its normal size; it was hard, nodular and yellowish-white. At the base of the gland the capsule was apparently invaded. A few lymph nodes in the right pelvic and right para-aortic chain were moderately enlarged and grayish-pink. The bone showed no changes.

Microscopic Observations.—These confirmed the gross observations. The tumor was made up of closely-packed large polyhedral cells with finely granular cytoplasm and large vesicular hyperchromatic nuclei. These were arranged in poorly formed acini, cords and clusters. The stroma was loose, abundant and cellular. There was little mitotic activity. The lymph nodes showed partial replacement with tumor tissue.

This prostatic carcinoma showed the most extensive and constant perineural invasion.

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12. Ferguson, R. S.: Am. J. Cancer **16**:783, 1932.

The large sections showed the prostate diffusely invaded by the tumor, as also its capsule, and in the latter there was extensive and striking perineural invasion. The posterior wall of the bladder below the ureteral orifices was fairly widely invaded, but infiltration above the ureters faded out fairly rapidly. The proximal parts of the ureters and vasa were invaded for a short distance. The seminal vesicles showed much invasion, and in the tissue about them were many cords of the tumor, ranging up to 2.5 mm. in diameter. The muscularis and submucosa of the rectum were extensively invaded. No tumor was present in the sigmoid or in the upper part of the rectum. A fairly large amount was present in lymphatics adjacent to the rectum, and much of this was perineural. In the sections with prostate and vesicles the greater part of the tumor was anterior, but in those above the prostate it was posterior to the rectum, and in some sections tumor-replaced lymph nodes were seen here. This tumor-infiltrated tissue extended unbroken up the sacrum, and everywhere perineural invasion was very extensive. The tumor also spread laterally but much less conspicuously until the lymph nodes beside the hypogastric and iliac vessels appeared. These were widely invaded. Sections higher up showed more invasion in the surrounding tissue, and here, too, perineural advance was very striking. This could be traced to the aorta. The periosteum in most of the sections contained masses of the tumor (some perineural), and in some places the tumor extended directly into the bone. In a few foci the tumor extended into the bone from about vessels in the outer part of the periosteum. The bone contained much neoplastic tissue.

CASE 5.—An American mechanic, aged 71, admitted April 15, 1933, had suffered a sudden onset of symptoms of obstruction of the bladder six weeks before. He had lost 25 pounds (11.3 Kg.). He was feeble, with evidence of marked loss of weight. The kidneys were palpable but not tender. The prostate was large and hard, with the induration extending into the region of the seminal vesicles beyond the reach of the examining finger. Cystoscopy revealed that the trigon was irregular and elevated, the ureteral orifices normal, and the region of the left margin of the internal sphincter edematous and ulcerous.

Roentgenologist's Reports.—"April 18: The pelvis and chest show no evidence of metastatic malignant growth."

"May 2: The lumbar spine shows pronounced hypertrophic changes but no definite evidence of metastases."

"July 21: A roentgenogram of the pelvis taken post mortem shows a very extensive metastatic process involving chiefly the pubis but also all the bones of the brim of the pelvis. There are also beginning changes in the upper end of the right femur. I do not believe that there is much involvement of the lumbar or of the dorsal spine."

Course.—The patient gradually failed, and marked signs of uremia developed. He died July 21. The interval between the onset of symptoms and the discovery of metastases at autopsy was about five months.

Autopsy.—The final diagnosis was: carcinoma simplex of the prostate with extension to the bladder; metastasis to the lungs, the liver and the inguinal, pelvic, para-aortic and abdominal lymph nodes, including the nodes at the hilus of the liver; bronchopneumonia; pyelonephritis and pyo-ureter on the left; arterionephrosclerosis.

The body is that of a well developed but markedly emaciated elderly white man. The observations were essentially unimportant except for the following: The liver weighed 1,740 Gm. and was thickly studded with yellowish-white nodules from 1 to 2.5 cm. in diameter. The right kidney weighed 120 Gm.; the left,

160 Gm. The right was essentially normal. The left showed poor demarcation of the cortex and prominent glomeruli. The pelvis was dilated and contained 10 cc. of cloudy urine. The left ureter was dilated to 0.6 cm. in diameter; the wall was thickened and indurated. The right ureter was not remarkable. The bladder contained 250 cc. of foul cloudy urine. The mucosa of the fundus showed marked injection. The floor was occupied by a fungating central tumor approximately 3 cm. in diameter, extending about 1 cm. above the mucosa. This was necrotic and ulcerated at the center; the margins were irregular. It was continuous with the prostatic mass. The prostate itself was considerably enlarged and firm, with the left lateral lobe very hard. The architecture was obliterated. The gland was markedly adherent to adjacent structures and encroached on the ampulla of the rectum. The inguinal, pelvic, para-aortic and all abdominal lymph nodes, including those at the porta of the liver, were enlarged, firm and on section yellowish-white and granular. Sections of the left ilium and lumbar vertebrae showed lipping at their margins but otherwise were not grossly remarkable.

Microscopic Observations.—These confirmed the gross observations except for the addition of early metastasis to the lung. The tumor was composed of strands and masses of poorly formed acini made up of acidophilic tumor cells varying greatly in size and shape, with finely granular cytoplasm and moderate-sized reticular nuclei. Mitotic figures were comparatively rare.

Perineural invasion in this case seemed less significant than in the other cases. The large sections showed widespread invasion of the prostate and its capsule, and in the latter perineural lymphatic invasion was seen, but the other lymphatics were more uniformly invaded and contained larger masses. The bladder was invaded, and in the posterior wall was a large polypoid mass (2 by 3 cm. in one section) which extended into the lumen. The ureters and vasa were not invaded. Some tumor was present in the periphery of the proximal part of the seminal vesicles. There was extensive invasion of the tissue between the prostate and the rectum, with formation of large neoplastic masses. One mass in the fat here measured 1 by 3 cm. The lymphatics here were much enlarged by the masses. Perineural lymphatic invasion was present, but the masses in these lymphatics were smaller than those in others, and there were many uninvolved nerves here. Perineural invasion was not seen at a distance from the large masses. The vasa and vesicles were in places surrounded by the tumor but not invaded. The anterior rectal wall was extensively invaded, and in one section the submucosa contained a mass measuring 1 by 2.5 cm. Large masses were present in the muscularis, too, but the tumor disappeared at the level of the lower end of the seminal vesicles. Several lymph nodes anterior and posterior to the rectum were replaced by the tumor. The lymph nodes beside the iliac vessels were invaded. There were numerous large tumor-replaced lymph nodes anterior to the sacrum and vertebrae, some lying against the bone. The sacrum and vertebrae showed much invasion, but, although there was much tumor on both sides of the periosteum, in many sections the periosteum contained no tumor. In some sections a small amount of the tumor was seen in lymphatics in the periosteum, and in three sections the periosteum contained more of the tumor, and direct extension into the bone was seen. A few small veins in and outside the periosteum contained tumor, and a rare example of perineural invasion was seen.

Case 6 (Carcinoma of the bladder included as a control).—An American millworker, aged 59, admitted Sept. 25, 1933, had experienced difficult urination, dysuria and increased frequency for about eight months. Suprapubic cystostomy was done two weeks before admission. He had a history of gonorrhea and syphilis. Wassermann and Hinton tests of his blood made at the Pondville State Hospital for

Cancer were negative. His weight was from 30 to 35 pounds (13.6 to 15.9 Kg.) below the usual average. He presented the appearance of anemia and loss of weight. The right kidney was felt faintly and was slightly tender. He had a catheter in a suprapubic sinus. The prostate was very slightly enlarged and benign. Cystoscopy disclosed foul urine, extensive ulceration and slough.

Roentgenologist's Reports.—"September 28: There is density of the bone of practically the entire right side of the pelvis with considerable thickening of the bone and increase in the prominence of the trabeculation. There is also definite increase in the density of the upper part of the left femur. Not much change is noted in the left iliac bone or in the spine. There is no change in the upper part of the right femur. The changes are almost characteristic of Paget's disease but conceivably could be due to metastatic disease. I should like to have roentgenograms of a few other bones. Those of the chest show no metastases, and there is no evidence of disease in the pulmonary fields."

"October 3: A lateral view of the skull shows a number of fairly well defined areas of increased density in the bones of the vault. The left femur presents expansion on the bone in the upper half of the shaft and rather coarse trabeculation. I believe this patient has Paget's disease. He may have metastatic malignant change of the bones of the pelvis in addition to Paget's disease."

Course.—The patient showed gradual failure in the next few weeks, and death occurred Nov. 2, 1933.

Autopsy.—The final diagnosis was: papillary epidermoid carcinoma of the bladder, grade 2, with extension to the perivesical soft tissues; pyelonephritis; aortic insufficiency; multiple colloid adenomatous goiter.

The postmortem observations were essentially unimportant except for the following: The body showed marked emaciation and an old suprapubic cystostomy opening in good condition. The right kidney weighed 160 Gm.; the left, 140 Gm. The capsule stripped with difficulty from an irregularly rough surface, leaving foci of yellowish-green necrotic material. Numerous foci of very irregular outline, up to 2 cm. in diameter, were scattered through the renal substance. The ureters were thick-walled, and each was dilated to about 0.4 cm. The mucosa was covered with yellowish-green exudate. The bladder was adherent to the anterior abdominal wall at the region of the cystostomy. The bladder was contracted. The anterior and left lateral walls were occupied by a large fungating ulcerated mass, roughly 9 cm. in diameter, extending from the internal sphincter almost to the apex. This tissue was soft and friable. On section it was pale red to yellowish white, granular and firm, and invaded the entire thickness of the wall of the bladder as well as the perivesical soft tissues. There were a few slightly enlarged para-aortic lymph nodes, which contained small yellowish necrotic foci. The bone was not involved except that the left femur showed some thickening of the cortex and fibrosis of the marrow cavity.

Microscopic Observations.—These confirmed the gross findings. The tumor consisted of coarse masses of irregularly shaped large cells supported on a loose vascular, rather papillary stroma. The cytoplasm was darkly stained, finely granular and often vacuolated. The nuclei were large, vesicular and hyperchromatic, with rare mitotic figures. There was marked necrosis.

In the large sections the anterior wall of the bladder was seen to be extensively invaded by the tumor, but the posterior wall contained very little of it. In three sections (nos. 5, 6 and 7) a few small masses were seen about the seminal vesicles, and in one of these a vein anterior to the rectum was occluded by a mass of tumor cells. The tumor was not seen elsewhere.

CASE 7.—A Canadian tailor, aged 58, admitted Aug. 8, 1932, gave a history of increased frequency of urination and retardation of the stream for six months. He had pain in the right flank and groin. He had lost from 12 to 15 pounds (5.4 to 6.8 Kg.). He had no pain in the back or legs. He was in good general condition. There was tenderness in the right renal area, and the kidney was faintly palpable. There was a hydrocele on the right. The prostate was moderately enlarged, firm and irregular. The upward extension was more marked on the right side. Cystoscopy revealed hypertrophy of the trigon and irregularity and marked edema of the margin of the internal sphincter, especially posteriorly.

Roentgenologist's Reports with the Intervening Treatment.—"August 15: An intravenous pyelogram indicates normal secretion of dye on the left side. The pelvis of the left kidney appears normal. There is practically no function on the right."

"September 2: The lumbar spine and pelvis show no evidence of metastatic malignant change."

(Roentgen therapy was applied—1,500 r each to the anterior and posterior regions of the pelvis. October 5 a perineal implantation of radium in the prostate was made [5,544 millicurie hours].)

"October 15: The pelvis shows the shadows of fourteen radium implants rather widely distributed."

"October 15: The lower half of the pelvis shows several additional radium implants. The bone of the right ischium appears slightly mottled. This is very suggestive of an early metastatic process."

(The patient was discharged to go home October 15. He was readmitted for high voltage roentgen therapy in February 1933—800 r to the anterior and posterior surfaces of the pelvis, respectively.)

"February 2: The pelvis shows slight extension in the process previously noted in the right ischium. The appearance is definitely that of a metastatic process. No other foci of disease appear in the pelvis."

(The patient was readmitted September 22. He had gained 13 pounds [5.9 Kg.]). A gradual return of urinary frequency had been noted in the past few months. There was a large fixed mass in the region of the prostate. The nonprotein nitrogen in the blood amounted to 150 mg. per hundred cubic centimeters. The urethral catheter drainage was constant. In October roentgen treatment was given—600 r to the anterior and posterior regions of the pelvis, respectively.)

"September 25: There is a slight extension of the process in the right ischium; possibly there are early changes on the left side. The other portions of the pelvis and lumbar spine do not show definite metastases."

"October 21: The diaphragm appears normal in position and outline on both sides. The pulmonary field on the right is of normal radiance; that on the left, of somewhat diminished radiance. There are no gross areas of consolidation."

Course.—The patient presented gradual failure with signs of pulmonary and renal infection and died December 10. The interval between the onset of symptoms and the clinical demonstration of metastases was eight months. The length of life after the demonstration of metastases was fourteen months.

Autopsy.—The final diagnosis was: adenocarcinoma of the prostate with metastasis to the lungs, liver and periureteral lymphatics; bilateral pyelonephritis; pyoureter on the left; hydro-ureter on the right; pyophlebitis; bronchopneumonia; tuberculosis of the lungs; hydrocele on the right.

The body was that of a fairly well developed but extremely emaciated elderly white man. The following postmortem observations were significant: The apexes

of both lungs contained scattered firm nodules. The spleen showed a firm nodule of poorly demarcated yellowish tissue with some central softening. The liver weighed 1,350 Gm. and contained numerous whitish or yellowish nodules from several millimeters to 0.5 cm. in diameter. Many were necrotic in the center; others were cystic and filled with thin bile-tinged fluid. The right kidney weighed 70 Gm.; the left, 260 Gm. The right kidney was essentially a large distended pelvis, thin-walled, containing 40 cc. of thin clear urine. The renal substance had been compressed to from 2 to 4 mm. in thickness. The calices were markedly distended. The left kidney was about twice the normal size. The capsule was adherent and when stripped away left a pale, coarsely granular surface. There was poor differentiation between the cortex and the medulla. Numerous abscesses from 1 to 2 mm. in diameter were scattered through different parts of the renal substance. The calices were dilated, and the pelvis contained 35 cc. of thick creamy pus. Both ureters were dilated, the left to 1 cm. in diameter and the right to 0.7 cm. The walls were thickened, and scattered along their course were numerous nodules composed of homogeneous gray firm material. The bladder was small; the walls, thickened. The mucosa was red and covered with flecks of fibrin. The trigon was prominent, and just inside the sphincter posteriorly was an irregular broad, flat elevation. The prostate was three times the normal size and densely adherent to the adjacent pelvic structures. The seminal vesicles could not be identified. The rectum was apparently not invaded. Section revealed numerous discrete grayish areas, their centers somewhat necrotic. The iliac and para-aortic nodes were enlarged, gray and firm. The bone grossly was uninvolved.

Microscopic Observations.—In some foci in the lungs the peribronchial lymphatics were invaded by cords and clusters of large polyhedral cells with darkly staining granular cytoplasm and large vesicular hyperchromatic nuclei. Mitoses were rare. The metastases in the liver were composed of similar cells. A section of the bladder contained no tumor. The prostate was diffusely invaded by tumor cells, and the normal acini were nearly entirely replaced.

In large sections fairly large masses of the tumor were present in lymphatics in the sphincter ani, and many lymphatics in the levator ani were distended by tumor to as much as 2 mm. in diameter. A few small masses were present in the submucosa. Lymphatics in the outer muscle of the rectum were invaded, in some places extensively and in others slightly. Small masses were also seen in the submucosa above the level of the prostate, but above the seminal vesicles the rectum was free from the tumor. The prostate was diffusely invaded by the tumor, and there was extensive invasion of the capsule by masses, some large. Perineural invasion in some places was abundant, and in some was not very striking. It did seem to be of real significance, however. There were fairly large masses between the prostate and the rectum, between the seminal vesicles and the rectum and about the vesicles. Perineural invasion here was fairly extensive but not constant. The bladder showed much invasion inferiorly and posteriorly and some in the right lateral wall. The ureters were not invaded. In one section the outer part of the seminal vesicle was invaded. The tumor was present in one section beneath the peritoneum of the pouch of Douglas, and there was much invasion of lymphatics about the right vas and ureter, but this faded out soon. Perineural invasion was fairly extensive here.

There was no tumor in any of the bones, but in some of the sections of the sacrum soft tissue anterior to the bone showed slight lymphatic invasion with some perineural involvement—sections 2 and 3 in a series of eleven bone sections—and in section 6 the tumor was seen in a small lymph node near the bone.

Case 8.—An American laborer, aged 64, admitted Jan. 27, 1934, had begun to complain of difficult voiding of urine, increased frequency and dysuria less than two months before. These difficulties culminated in complete retention. He also complained of severe pain in the chest radiating down the left arm, dyspnea and edema. There was no loss of weight. He was in poor general condition. The kidneys were neither palpable nor tender. The prostate was only slightly enlarged and of benign consistency. Cystoscopy disclosed marked elevation in the posterior wall of the bladder, not suggestive of tumor in appearance, and very slight evidence of prostatic obstruction.

Roentgenologist's Reports.—"January 30: The pelvis shows some increase in the density of the bone in the region of the pubis and ischium on both sides. I suggest that a lateral view of the skull be taken before any conclusions are drawn."

"February 8: The lateral view of the skull shows calcification of the pineal body. There is no evidence of disease in the bones of the vault. The cystogram shows a defect in the right side of the bladder. I believe that the changes seen in the bone in the pelvis are due to Paget's disease."

Course.—The patient failed rapidly and was stuporous and irrational much of the time. There were severe pain in the chest and signs of cardiac decompensation. Suprapubic drainage was advised, but his condition would not permit operation. Finally bronchopneumonia developed, and death occurred March 11. The interval between the onset of symptoms and the discovery of metastases at autopsy was three and one-half months.

Autopsy.—The final diagnosis was: carcinoma simplex of the prostate with metastasis to the bladder, pelvic and para-aortic lymph nodes, vertebrae and lungs; hydronephrosis and pyelonephritis on the right; papillary adenoma of the kidney; hypertrophy and dilatation of the heart; congestion of the lungs; pleural effusion; arteriosclerosis; duodenal ulcer; bronchopneumonia.

The significant postmortem observations were: The pleural cavities contained 500 cc. of fluid. There were moderate cardiac hypertrophy and a bleeding duodenal ulcer. The right kidney contained two abscesses, and the right ureter was slightly dilated. The left kidney and ureter were normal. The wall of the bladder was much thickened, and the mucosa was ulcerated and covered by greenish-black slough. Posterior to the internal urinary meatus was a fold of mucosa 1 cm. high fixed to the prostate and extending transversely across the bladder. The prostate was 5.5 cm. in diameter, very hard and nodular, but not firmly adherent to the surrounding structures. The cut surface was yellowish white and revealed invasion of the posterior part of the capsule. The para-aortic and pelvic lymph nodes were all enlarged, hard and adherent to surrounding structures. The lower abdominal nodes formed a large mass on each side of the aorta. The bodies of all vertebrae from the eighth dorsal to the fifth lumbar were replaced by the tumor.

Microscopic Observations.—The tumor was made up of small clumps of closely packed epithelial cells with oval vesicular nuclei. Alveolar formation was rare. There were occasional mitotic figures. Numerous clusters of tumor cells were present in lymphatic spaces of the musculature of the bladder. There were small foci of tumor in the periprostatic fat.

Large sections showed the tumor present in the bladder posteriorly near the prostate. All sections of the prostate showed extensive invasion with much invasion of lymphatics in the capsule. There was much perineural lymphatic invasion also. In one section a few veins were invaded. The rectum and sigmoid contained no tumor, and there were no masses between the prostate and the rectum. The ureters, vasa and seminal vesicles contained no tumor. There were no masses

outside the prostate. Many normal nerves and ganglions were seen lateral and posterior to the seminal vesicles. A few small normal lymph nodes were seen here. Some sections of soft tissues above the bladder contained no tumor. In one, a small amount of the tumor was seen in lymphatics about a nerve trunk posterior and lateral to the seminal vesicle. Several sections included greatly enlarged lymph nodes, nearly or completely replaced by the tumor, beside the iliac vessels. In a few, the tumor was also seen in a few lymphatics outside the nodes. One node in front of the sacrum contained the tumor, as did some lymphatics in the adjacent muscle.

No invasion was seen in the coccyx. A section of the ischium did contain the neoplastic tissue, and neoplastic growth was present in the adjacent soft tissue. Some sections of the sacrum contained no tumor, but others contained much. Within the periosteum of the upper half of the sacrum were many small masses, some perineural. Many small masses were also present just outside the periosteum, and here, too, much of the tumor was perineural. Direct invasion of the bone was seen in several places.

COMMENT

These cases stress the importance of dissemination of prostatic carcinoma by the perineural lymphatic spaces. In all there was perineural lymphatic invasion. In the control case of advanced carcinoma of the bladder, no perineural invasion was found.

Perineural lymphatics may be infiltrated and spread of tumor occur by permeation with maintenance of continuity or, rarely, as in case 3, by lymphatic embolism. It may be objected that this perineural involvement in our cases is a late development due to widespread disease. Its occurrence, however, in early cases is well established. In practically all the cases which we have studied, entirely aside from those in this series, perineural lymphatic involvement was an important factor. At times diffuse involvement of the nerve and surrounding tissue occurred.

One also receives a certain amount of indirect evidence bearing on the involvement of the nerves from the frequency with which pain appears as an important symptom of prostatic cancer. Whereas hypertrophy of the prostate produces, as a rule, more obstruction to urinary outflow and more pressure on surrounding structures, nevertheless associated local pain is infrequent. With carcinoma of the pancreas, carcinoma of the breast and carcinoma of the skin, for example, pain is not a prominent feature until the tumor has reached a formidable extent. On the other hand, pain is common in cases of carcinoma of the prostate.

This involvement of the perineural lymphatics is not a peculiar property of prostatic cancer, but a prominent feature because of the unusually abundant nerve supply in the prostate, its capsule and the adjacent tissue. Thus in an epidermoid carcinoma of the tongue, an organ where the nerve supply is abundant, one not rarely sees extension of the tumor along perineural lymphatics.

These periprostatic nerves are distributed widely, some running laterally toward the iliac vessels and coming in close proximity to the

bony pelvis and another group passing along the perirectal soft tissues to the sacrum and lower part of the lumbar spine.

The lymphatic channels from the prostate do not come into as intimate contact with bone as do the nerves with their accompanying lymphatics, so that involvement of bone from regional or distant lymph nodes and their related lymphatic trunks would be more unlikely.[13]

It will be noted in the description of the slides studied that very little evidence was found of involvement of nerves or of their lymphatics extending toward the bony pelvis. This was more or less to be expected in view of the orientation of the section in the horizontal plane, which made laterally spreading lymphatics or nerves much less likely to be seen than had they been running at right angles to the plane of section.

We wish to emphasize that true hematogenous bony metastases are present and that the perineural lymphatics offer the tumor an additional way of access to bone, which accounts for the marked frequency with which the pelvis and the lower vertebrae become involved as compared with regions more distant from the tumor.

We may regard, then, the perineural lymphatics as pathways leading the cancer into intimate contact with cortical bone. Thence growth through the ostia is relatively simple, and invasion of the bone follows. In our sections, as may be noted from the descriptions, direct invasion of bone has been repeatedly demonstrated. Its detection by roentgen examination is much more difficult and probably impossible. It was not made out roentgenographically in any of the present cases. However, figures 9, 10 and 11 of Graves and Militzer [10] show a sequence of roentgenograms which strongly suggest direct invasion of bone from without, sweeping across the vertebral body.

Several interesting points with regard to roentgen diagnosis of bony metastasis of prostatic cancer developed: It has long been recognized that metastases of carcinoma of the prostate may give symptoms before any roentgen evidence of their existence can be obtained. This study brings out a possible explanation. Obviously, if the tumor is advancing along perineural lymphatics and developing in the lymphatic plexus in close contact with the periosteum and cortical bone, as our sections demonstrate, there will be a period when there is a considerable amount of pain from periosteal involvement without definite disturbance of the bone itself. Only with invasion of the ostia and growth into marrow spaces with induction of changes in the bony trabeculae will there be changes demonstrable by roentgenogram. Just as pain is an early symptom of primary carcinoma of the prostate, so it may be an early symptom of its metastases.

13. Llorca, F. O., and Botár, J.: Ann. d'anat. path. **10**:37, 1933.

The osteosclerotic nature of the metastases of prostatic carcinoma has long been a cause for speculation. Our present study sheds no light on the reason for this type of reaction in bone, but the assumption is justified that the slow growth of the metastases permits the pressure of the growth to stimulate osteoblasts to increased activity rather than to destroy those osteoblasts and their products.

Case 1 of this series is of particular importance in that it emphasizes a second source of error in roentgen diagnosis of metastases of carcinoma of the prostate. Not only may a metastasis be missed, but also, as in this case, a region of altered density in bone may be falsely interpreted as a metastasis. Some of the apparent pelvic metastases in case 1 were shown to be present by microscopic examination. Other apparent metastases, and the lesions in the axial skeleton, were found not to be metastases but merely regions where there was variation in the thickness of the bony trabeculae and in their frequency. This variation may possibly represent an irregular resorption in a process of osteoporosis. There was no fibrosis to suggest the healing of a previously existent carcinomatous metastasis but merely normal fatty marrow and thin, somewhat infrequent trabeculae.

Aside from the bony metastases, certain points regarding the extension of the tumor in the adjacent soft parts were brought out by this study and will be made the subject of a later report. Invasion of the bladder was frequent and extensive. The supporting structures about the seminal vesicles, the vasa and the ureters were frequently involved in the tumor, but actual invasion or replacement of these structures by tumor was extraordinarily rare.

Of particular interest was the high proportion of cases in which the rectum was involved. Here the perineural lymphatic pathway was an important factor in conducting the tumor from the periprostatic tissues into the rectal tissues. In several instances tumor cells could be demonstrated in the region of the myenteric plexus.

SUMMARY AND CONCLUSIONS

Seven cases of carcinoma of the prostate and one of carcinoma of the bladder were studied by means of large sections through the soft tissues and adjacent bones of the pelvis. Involvement of perineural lymphatics was a constant feature in the cases of carcinoma of the prostate and was absent in that of the carcinoma of the bladder. Owing to the abundant nerve supply in and about the prostate, opportunity for involvement of perineural lymphatics is great. The pain associated with prostatic cancer is clinical evidence of the existence of perineural involvement.

While hematogenous metastasis may occur in carcinoma of the prostate, the chief factors in the marked preponderance of metastatic lesions in the pelvis and in the lower regions of the spine are the distribution of the nerves and the conduction of the tumor cells along the perineural lymphatic spaces into close contact with the bone.

Metastasis often occurs as a direct invasion of the marrow spaces through the cortical ostia.

Early metastases may be missed in roentgen study. Conversely, under rare conditions there may be erroneous diagnosis of metastases, when there actually is only an irregularity in the extent of bone resorption.

Invasion of the lymphatic channels is frequent, and metastasis by the usual lymphatic route is an important factor in the spread of the disease, although probably not important in determining the localization of the bony metastases.

The perineural lymphatic dissemination of the tumor may be either by continuity or through embolism.

Involvement of soft parts, particularly the rectum, seminal vesicles, vasa and ureters, can be readily studied by this method and will be reported on at a future date.

THE NATURE OF EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS IN THE RABBIT

G. LYMAN DUFF, M.D. PH.D.
TORONTO, CANADA

Experimental investigations of arteriosclerosis in animals have for many years attracted an interest which has been sustained and stimulated not only by the complexity of the problems involved but by the far-reaching importance of the disease in human beings. Commencing in 1903 with Josué's discovery of the deleterious effects of injections of epinephrine hydrochloride on the arteries of rabbits, there appeared a flood of publications reporting the results of experimental researches on arteriosclerosis, and this has continued, with minor fluctuations of enthusiasm, up to the present time. In recent years, the great majority of these researches have been concerned with experiments on cholesterol feeding in rabbits, the results of which have been given special prominence because of their great importance in relation to current theories of the etiology and pathogenesis of arteriosclerosis in man. The results of all these experiments on the feeding of cholesterol-rich diets to rabbits and other animals have been reviewed in detail in a separate publication.[1] For the purposes of the present paper, therefore, it will suffice to mention the pertinent information derived from previous investigations and subsequently to indicate certain fallacies of current interpretation which should be brought to attention.

During the past twenty-five years it has been demonstrated repeatedly that the administration of cholesterol-rich diets will produce in rabbits abundant accumulations of doubly refractive lipoids in various organs and will lead finally to the development of widespread lesions in the arteries. This experimental disease of the arteries I have called "experimental cholesterol arteriosclerosis." Efforts to produce experimental cholesterol arteriosclerosis in animals other than rabbits have met with very limited success. It has been shown that the disease can be produced in guinea-pigs, though with considerably greater difficulty than in rabbits, but the results obtained in other rodents up to the present time have been equivocal. In cats, dogs and monkeys all

From the Department of Pathology and Bacteriology, University of Toronto.

1. Duff, G. L.: Experimental Cholesterol Arteriosclerosis and Its Relationship to Human Arteriosclerosis, Arch. Path. **20**:81 and 259, 1935. (Through an editorial error, reference 45 in the bibliography of this review was printed erroneously. The reference was intended to indicate the present paper.)

attempts to produce arterial lesions by cholesterol feeding have invariably met with failure, for these animals are adequately equipped to dispose of excessive quantities of cholesterol introduced in the diet. Thus, among the mammals which have been studied the rabbit is unique in its extraordinary susceptibility to the effects of cholesterol feeding, and consequently the investigation of experimental cholesterol arteriosclerosis has been confined almost exclusively to this animal.

Experimental cholesterol arteriosclerosis in rabbits has attracted a great deal of attention because of the general resemblance of the arterial lesions to the familiar lesions of arteriosclerosis in man. This resemblance is admitted by all, but some investigators, apparently in the attempt to emphasize the similarity and perhaps in order to support their own ideas of pathogenesis, have given incomplete and even misleading descriptions of the experimental lesions of the arteries, disregarding certain variations which undoubtedly occur. The development of the arterial changes is generally described as a perfectly regular process commencing with the appearance of fatty droplets in the intima. It is implied that the intimal changes are always primary and always the most conspicuous. On the other hand, changes in the media, if mentioned, are described only with the greatest reluctance and so with extreme brevity. Frequently a specific reference to alterations in the media appears to be avoided deliberately by the ambiguous statement that "the lesions always involve the innermost layers of the arterial walls." The intimal changes are then discussed at length.

Careful examination of the descriptions given by various investigators shows that the development of experimental cholesterol arteriosclerosis is not always the same and that medial involvement, while not a constant occurrence, is not infrequent. It is not my intention to suggest that the lesions in the media are in any way responsible for the development of those in the intima. Either intimal or medial changes may appear first, and their close association suggests an identical etiology for both. The lesions of the media, however, are of importance in that they furnish additional information which has a direct bearing on the pathogenesis of the experimental arterial lesions. The following experimental observations will suffice to illustrate the character of the medial lesions, as well as certain variations which occur in the lesions of the intima, and will serve to indicate some directions in which the current interpretations must be modified.

EXPERIMENTS

In this series of experiments twenty rabbits were used, all of which were less than 6 months old. They were divided into four groups, each composed of five animals. The animals of three groups were given daily feedings consisting of egg yolks or a 3 per cent solution of cholesterol in olive oil, or a combination of

both. In the fourth, or control, group, two animals were given daily feedings of pure olive oil, while the others were kept on their usual diet. The feedings in all cases were administered through an ordinary soft rubber catheter used as a stomach tube. All the animals were allowed their usual ration of oats, carrots and greens in addition to the special feedings. The details of the experiments are shown in the table.

The experiments of less than twenty days' duration were terminated by the spontaneous death of the animals due to diarrhea, which commenced one or sometimes two days before death. This occurred only in animals of the second and third groups, which were receiving olive oil as part of their feeding. The two controls which were receiving olive oil also died as a result of diarrhea after relatively short periods of feeding. The remaining animals of both the experimental groups and the control group were maintained in apparently good health and were killed at the end of the experimental periods by the intravenous injection of air.

Details of Experiments

Group	Rabbit	Daily Feeding in Addition to Normal Diet	Total Number of Feedings	Duration of Experiment, Days	Lipoid Deposits in Aorta
I	1	1 egg yolk	20	25	None
	2		72	87	Microscopic
	3		83	100	Gross
	4		86	105	Gross
	5		100	119	Gross
II	6	1 egg yolk + 5 cc. of a 3% solution of cholesterol in olive oil	11	12	None
	7		20	25	Microscopic*
	8		21	27	None
	9		80	97	Gross
	10		92	110	Gross*
III	11	10 cc. of a 3% solution of cholesterol in olive oil	7	8	None
	12		11	14	Microscopic*
	13		14	18	None
	14		20	26	None
	15		32	38	Microscopic
Control	16	10 cc. of pure olive oil	8	10	None
	17		21	25	None*
	18	None	—	87	None
	19		—	97	None
	20		—	119	None

* The aorta of this animal showed scattered spontaneous lesions of the media and will be described separately.

Autopsy was performed as soon as possible after death. The aorta and all the organs were carefully examined grossly and then preserved in a 10 per cent dilution of solution of formaldehyde U. S. P. Paraffin sections of the organs and the aorta were stained with hematoxylin and eosin, and those of the aorta were also stained with Verhoeff's elastic tissue stain and various other special stains. Frozen sections were stained with sudan III and hematoxylin stains and were examined with polarized as well as ordinary light. When no gross lesions were present in the aorta, sections were taken from representative areas, two in the arch, two in the thoracic portion and one in the abdominal portion. When gross or microscopic lesions were noted, numerous sections were taken from all parts of the aorta and also from the pulmonary artery.

OBSERVATIONS

In the animals of the three experimental groups the effects of the three different cholesterol-rich diets were not perceptibly different. Gross and microscopic examination of the organs showed a progressive accumulation of lipoids in the adrenal cortex, liver, spleen, kidneys and elsewhere. These accumulations have been described by numerous investigators whose results have been well summarized by Schönheimer.[2] A detailed account of the changes in the organs is not essential in the present paper, and their description will be omitted. Lesions were observed in the aorta in a sufficient number of animals to permit of examining them at various stages of advancement. The lesions are described in the following paragraphs, the findings in rabbits 7, 10 and 12 being omitted for the present.

The earliest appearance of lesions in the aorta in this series of experiments was in rabbit 15, which had been fed cholesterol in olive oil for a period of thirty-eight days. The aorta showed no lesions on gross examination, but microscopic changes were noted in sections of the arch. The remaining parts of the aorta showed no histologic abnormalities. Study of sections from the arch of the aorta showed no change in the intima, but the inner layers of the media contained groups of lesions consisting of small areas of necrosis in which the muscle fibers had been lost, leaving only occasional fragments of cells and a few pyknotic nuclei scattered at wide intervals through a pale-staining, cloudy or flocculent material which occupied the areas of muscle destruction. This material stained lightly with all the stains that were used and showed no definite architecture except in a few instances, in which there was a suggestion of a fibrillar structure arranged in the form of a fine network. Weigert stains for fibrin gave negative results. With Verhoeff's elastic tissue stain the individual medial lesions were seen to be sharply limited by the elastic laminae on either side, and at the ends by elastic fibrillae crossing between the adjacent laminae, so that the individual areas were usually elongated or fusiform in outline. The areas varied somewhat in size, the largest being noted toward the center of groups of such lesions and the smallest at the periphery. Sections stained for fat revealed the presence of numerous minute granules or droplets of anisotropic lipoid material strewn through the flocculent ground substance which remained in the larger areas of muscle destruction. The smaller lesions around the periphery contained no stainable lipoids (fig. 1). Occasional small wandering cells containing a few minute granules of fat were seen in the media, near the areas occupied by the accumulations of lipoids. The intima was totally free from

2. Schönheimer, R.: Virchows Arch. f. path. Anat. **249**:1, 1924.

sudanophil material. No similar lesions of the media, either with or without fat, were to be observed in the aortas of the control animals.

Rabbit 2, which had received seventy-two egg yolks in eighty-seven days, showed lesions of the same kind but much more severe. No lesions were visible on macroscopic examination of the aorta, but sections of the arch that were stained for fat, while showing no intimal change, revealed denser accumulations of droplets of anisotropic lipoid in the inner layers of the media. The fatty material lay between elastic fibers in areas of necrosis occupied by a flocculent ground substance identical in appearance with that noted in the experiment of shorter duration. Many of the elastic fibers bordering on these lesions showed

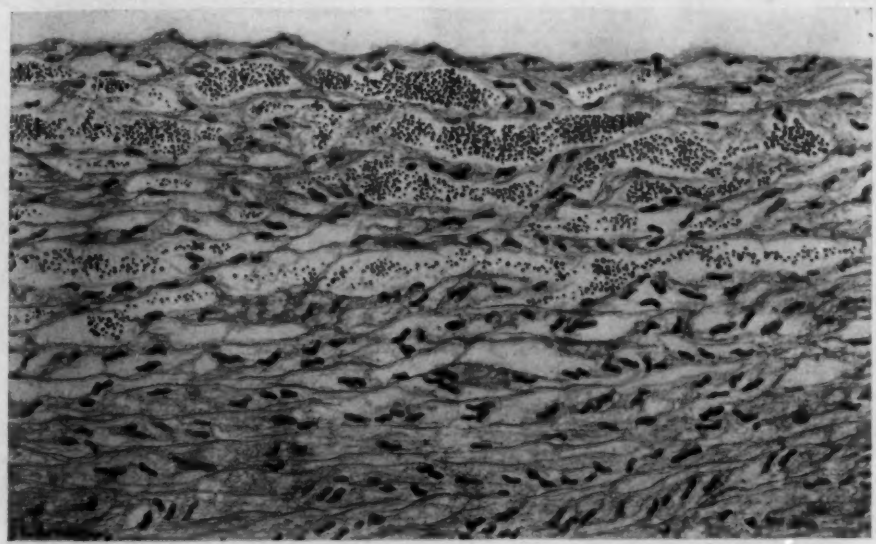

Fig. 1 (rabbit 15).—Camera lucida drawing of the inner part of the wall of the vessel in a frozen section of the arch of the aorta, showing the pale-staining anuclear areas of muscle destruction in the media. The small areas of necrosis contain no stainable fat, but in the larger areas the flocculent ground substance is filled with minute droplets of anisotropic lipoids represented in the drawing as black dots. The intima shows no change from its normal appearance. Sudan III and hematoxylin stains; × 200.

evidence of degeneration in the form of fraying and splitting, and some of them showed actual breaks of continuity. In this animal, too, although the fatty accumulations were larger, more numerous and dense, the smaller areas of medial necrosis about the edges of groups of lesions were, as yet, free from lipoid infiltration.

The same animal (rabbit 2) showed also the earliest deposits of lipoid in the intima, but not in the arch where the medial lesions were

seen. The intimal changes were confined to the upper part of the thoracic aorta. In this region microscopic examination revealed the presence of numerous tiny droplets or granules of anisotropic lipoids, which were scattered in small groups here and there in the subendothelial layer of the intima. The total quantity of lipoid material was not great, but all of it lay extracellularly in the subendothelial ground substance. The latter was swollen in appearance, and this swelling had raised the lining endothelium from its usual position close to the internal elastic lamina not only where the deposits of lipoid were observed but also for a short distance on each side. There was as yet no cellular proliferation in the intima. The media showed no abnormalities in this region.

In all of the animals that had been fed for ninety-seven days or longer, fatty lesions of the aorta were visible on gross examination. The smallest of these appeared as small discrete rounded pale yellow thickenings on the intimal surface. The larger lesions were thicker and more irregular in their outline, often suggesting the confluence of several smaller plaques. In the most advanced case (that of rabbit 5), in which feeding had been continued for one hundred and nineteen days, the arch of the aorta showed hardly any areas of normal appearance. The intimal surface was roughened by rounded yellowish thickenings which almost everywhere coalesced one with another.

The distribution of the lesions on the intimal surface of the aorta corresponded closely with that described by many investigators. The lesions developed most rapidly in the arch and were always most advanced in that situation. They tended to occur in the areas above the anterior, and left posterior sinuses of Valsalva and in the convexity of the arch where the fatty plaques sometimes completely surrounded the openings of the great vessels. In the descending limb of the arch the lesions were more numerous and farther advanced on the posterior surface; this was also the case throughout the thoracic aorta. The intimal thickenings were frequently noted near or even surrounding the mouths of the branching vessels in both the thoracic and the abdominal portions of the aorta. However, they were not confined to these areas, and the distribution of the lesions was never exactly the same in different aortas. In the pulmonary artery the lesions had the same gross appearance, but no characteristic distribution was established.

Some of the smaller lesions which were visible on gross examination consisted of intimal thickenings composed almost exclusively of a greatly swollen subendothelial layer impregnated with anisotropic lipoids and practically free from cells except for a few spindle-shaped or branched connective tissue cells (figs. 2 *A* and 3). The swollen subendothelial ground substance was pale-staining and homogeneous, but in paraffin sections of some of the lesions this subendothelial layer contained many

slitlike clefts suggestive of the presence of cholesterol crystals (fig. 2 *A*). Other intimal lesions of about the same size contained many large oval or polygonal cells filled with lipoid material (fig. 2 *B*). The variation in the number of these large cells in different intimal plaques was striking, as is illustrated in the contrast shown between figures 2 *A* and 2 *B*. However, in the larger intimal lesions cells of this type almost always made up the greater part of the intimal thickening. The large fat-containing cells, which are often referred to as foam cells, varied somewhat in size, but all possessed a rather voluminous cytoplasm, which in paraffin sections showed a distinctive vacuolated, foamy appearance. The nuclei were centrally placed, round, small and deeply staining. None of the intimal lesions contained any fibrin.

Staining for fat with the sudan III stain demonstrated the presence of large quantities of fatty material in the intimal thickenings. The

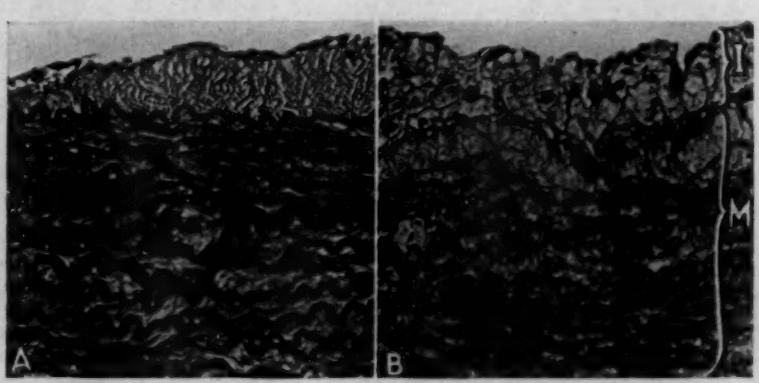

Fig. 2 (rabbit 3).—Photomicrographs of paraffin sections of the aorta. *A*, an early intimal lesion in the thoracic aorta, showing a greatly thickened subendothelial layer practically lacking in cells. The spindle-shaped nuclei of several connective tissue cells are visible, but foam cells are absent. The intercellular ground substance shows many slitlike clefts suggesting the presence of cholesterol crystals. In sections of the same lesion stained for fat the intima was seen to be densely impregnated with anisotropic lipoids, but the media was free from fat in this area. Verhoeff and Van Gieson stains; × 200. *B*, a lesion in the arch of the aorta, involving both the intima and the media. The intima is packed with lipoid-filled macrophages or foam cells. Pale-staining areas of necrosis in the inner layers of the media are clearly shown. Sections of the same lesion stained for fat showed abundant anisotropic lipoid material in the intima and the innermost zone of the media, but the smaller areas of necrosis in the media contained no fat. *I*, indicates intima, and *M*, media. Verhoeff and Van Gieson stains; × 180.

large foam cells were packed with minute droplets or granules of fat. Some of the fibrous connective tissue cells also contained a few droplets of fatty material in their cytoplasm. Large quantities of fatty

material in a finely divided state were found also in association with the subendothelial ground substance. Examination of frozen sections with polarized light showed that a large proportion of the fatty material, both intracellular and extracellular, was anisotropic.

In the more advanced intimal lesions the smaller foam cells and those nearest the surface of the intimal thickening were usually well preserved. However, the larger cells and those in the deeper parts of the plaque often showed degenerative changes. The nucleus gradually became paler-staining and finally underwent complete karyolysis. At the same time the outline of the cells became less and less distinct until disintegration was complete. The deeper parts of the intimal plaques thus came to be composed of an almost homogeneous mass of pale pink-staining anuclear débris in which numerous slitlike clefts suggested the presence of cholesterol crystals. In frozen sections these areas were seen to be packed with masses of anisotropic fatty granules and lipoid crystals.

With the commencement of necrosis of the large foam cells, fibrous tissue cells became more prominent. They usually made their appearance first in the deepest part of the intimal plaque in the form of long spindle-shaped cells. They sometimes lay parallel with the internal elastic lamina; in other places their direction was vertical to it, and they appeared to be spreading out into the area of thickening; in other places their arrangement was entirely haphazard. Some of them contained a considerable amount of fat in their cytoplasm. While the fibrous connective tissue cells were increasing in number, fibroglia fibrils and reticulum also became more abundant. A few fine elastic fibrils were also seen. In some places these appeared undoubtedly to be newly formed, but some of them showed a connection with the internal elastic lamina. In the latter case some fibers which at first sight appeared to be recently laid down were seen on closer examination to be thin shreds split off from the degenerating internal elastic lamina.

In none of the intimal thickenings were smooth muscle cells to be observed. However, when the internal elastic lamina had completely disintegrated a few surviving muscle cells of the media might be seen in the deepest layers of what then appeared to be intima.

The media of the aorta in these more advanced cases showed the same type of lesion as has already been described as occurring in two shorter experiments (figs. 3, 4, 5 and 6). The accumulations of anisotropic fat in the media were usually observed beneath plaques of intimal thickening and were more common in the arch of the aorta and in the pulmonary artery than elsewhere. However, early medial lesions were sometimes noted beneath an intimal layer which showed no change from its normal appearance, and, conversely, the media was sometimes normal beneath early intimal lesions.

In comparison with the lesions observed in the media in experiments of shorter duration, the medial fatty accumulations in the longer experiments were more abundant and extensive. However, in the peripheral parts of the medial lesions there were always to be observed small areas of necrosis in which there remained only a few fragments of cells strewn through the flocculent ground substance and in which the deposition of lipoids had not yet occurred. The medial lesions often showed such an abundant deposit of lipoids that the structure of the media in those areas was completely masked in sections stained for fat (fig. 4). In paraffin sections the areas in the inner layers of the media which had been occupied by lipoid materials showed widespread destruction not only of the muscle cells but also of the elastic laminae. Only a few

Fig. 3 (rabbit 4).—Paraffin section of the arch of the aorta. The intima shows a greatly thickened subendothelial layer in which only occasional connective tissue cells are to be seen. Foam cells are lacking. Areas of necrosis in the media are plainly visible, occupied only by pale-staining flocculent ground substance with scattered nuclear fragments. Sections of the same lesion stained for fat showed dense deposits of anisotropic lipoids in the intima and largest areas of medial necrosis. The smallest areas of necrosis in the media contained no fat, while those of intermediate size showed slight or moderate impregnation of the ground substance with lipoids. *I* indicates intima, and *M*, media. Verhoeff and Van Gieson stains; × 200.

fragments of the latter and a few distorted muscle cells remained, scattered through large "lakes" of pale-staining flocculent ground substance. The elastic laminae bordering on these areas did not stain smoothly with the elastic tissue stain but showed a granular appearance and were

often frayed and fragmented (fig. 5). The internal elastic lamina resisted destruction for a longer time, although the elastic fibers immediately beneath it were completely destroyed, but it, too, eventually disintegrated so that its position could be recognized only by the persistence of a few small fragments (fig. 6).

As in the shorter experiments, a few small mononuclear wandering cells were scattered through the affected parts of the media. Some of them contained a few granules of fat in their cytoplasm. These cells never reached the size of the large fat-containing cells observed in the intimal thickenings, nor did their cytoplasm show the same foamy appearance. Their numbers were few, and they contained only an insignificant proportion of the total quantity of lipoids in the media, almost all of which was extracellular. The accumulation of large foam

Fig 4 (rabbit 9).—Frozen section of the arch of the aorta, stained with sudan III and hematoxylin and photographed with a blue filter so that the lipoids show up as dark masses. The extent of the lipoid deposits in the media is shown in relation to the degree of intimal thickening. *I* indicates intima; *M*, media, and *A*, adventitia; × 100.

cells so characteristic of the more advanced lesions in the intima was entirely lacking in the media. However, as soon as a break appeared in the internal elastic lamina, numbers of large foam cells could be noted in the media near the defect. It appeared that these cells had migrated from the intima into the media. With the further destruction of the internal elastic lamina and the increasing abundance of foam cells in the inner layers of the media the latter finally assumed an appearance almost indistinguishable from that of the intima itself.

In none of the animals of the control group were any lesions or abnormalities observed in the organs. Four of the animals had perfectly

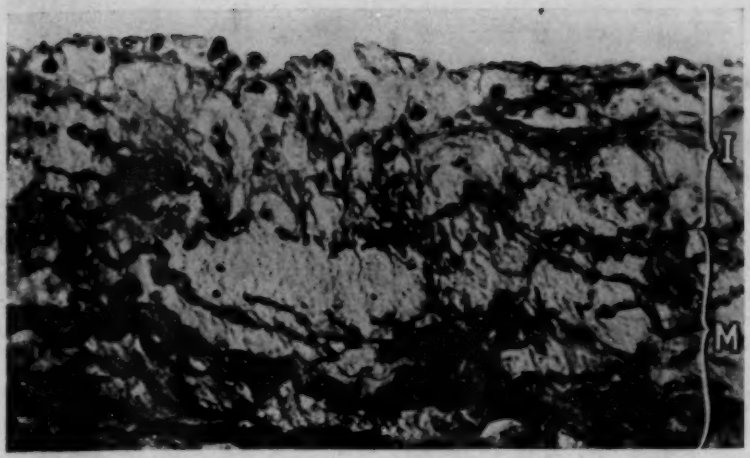

Fig. 5 (rabbit 9).—Paraffin section of the arch of the aorta. The elastic fibers around the lesions in the media show splitting and fragmentation. The internal elastic lamina has also undergone partial disintegration and is ruptured in the middle of the field. Both the intimal layer and the large medial lesions were seen to be densely impregnated with anisotropic lipoids, but foam cells, though abundant in the intima, have not invaded the media. *I* indicates intima and *M*, media. Verhoeff and Van Gieson stains; × 400.

Fig. 6 (rabbit 5).—Paraffin section of the arch of the aorta. The intima shows a thick plaque traversed through the midzone by a band of fibrous connective tissue in which fine elastic fibrils are visible. Foam cells are abundant on both sides of this band. Extensive medial lesions are shown as pale anuclear areas occupied only by flocculent ground substance. The elastic fibers between these areas are destroyed for the most part. The internal elastic lamina is intact in the right half of the field, but toward the left it has disintegrated, and the adjacent medial lesions contain scattered foam cells. Sections of the same area stained for fat showed dense accumulations of anisotropic lipoids in the intima and in all but the smallest of the medial lesions. *I* indicates intima; *M*, media., and *A*, adventitia. Verhoeff and Van Gieson stains; × 150.

normal aortas, and staining for fat with sudan III failed to reveal any trace of lipoid deposit in them. However, in spite of the precautions taken in using only young rabbits, one of the animals in the control group (rabbit 17), as well as three in the experimental groups (rabbits 7, 10 and 12), showed the presence of scattered spontaneous medial lesions of the aorta. Such lesions are not uncommon in rabbits, and those which were noted in the one control animal were typical of similar lesions which I have observed repeatedly in other rabbits. These lesions of the aorta in rabbits which have not been fed cholesterol appear in the gross specimen as small rounded depressed whitish areas on the intimal surface. Microscopically, the media is seen to be somewhat thinned out in the affected areas. The elastic laminae are straight and lie close together in parallel rows, the intervening muscle cells having disappeared, leaving only thin lines of homogeneous ground substance with occasional wandering cells between the elastic fibers. The latter may be more or less completely calcified. The intima overlying the medial lesions generally shows no proliferation, but the lining endothelial cells are slightly swollen, and there is considerable swelling of the subendothelial ground substance so that the lining endothelium is raised somewhat from its usual position close to the internal elastic lamina. Although the one control animal in which such lesions were observed had been given daily feedings of olive oil for twenty-five days, frozen sections stained for fat showed complete absence of material stainable with sudan III, not only in the spontaneous medial lesions but also in the remaining parts of the aorta. Absence of sudanophil material has been the rule in the spontaneous aortic lesions studied in numerous other rabbits.

On the other hand, in the three animals mentioned which had been fed cholesterol accumulations of lipoid material rapidly appeared in the aorta in relation to the spontaneous medial lesions. The deposits of lipoid in these situations appeared much earlier than elsewhere in the aorta and much earlier than in other rabbits which showed no such aortic lesions and which had been given a comparable diet.

The shortest experiment (that on rabbit 12) in which such an observation was made lasted only fourteen days. The aorta of this rabbit showed several pale, dimple-like depressions on the intimal surface of the arch, but no fat was visible on gross examination. In paraffin sections of these areas typical spontaneous medial lesions were observed. However, stains for fat showed the presence of a diffuse scattering of fine anisotropic fatty droplets deposited particularly in the altered ground substance between the slightly calcified fibers of the medial lesions. A few small mononuclear cells contained minute granules of lipoid material. The intima over these areas showed complete absence of stainable fat.

A similar but more abundant accumulation of lipoids was seen in the aorta of rabbit 7, to which a high cholesterol diet had been administered for twenty-five days. Several whitish areas of depression were seen on the intimal surface in the lower part of the thoracic aorta and in the abdominal portion. The vessel was somewhat thinned out in these regions, but no fat was visible to the naked eye. Microscopic examination revealed spontaneous medial lesions in these areas, which appeared typical in paraffin sections. In sections stained for fat, however, large numbers of minute droplets or granules of anisotropic and isotropic lipoids were seen lying between the elastic laminae in the homogeneous ground substance from which the muscle cells had disappeared. The fatty droplets were so minute and uniformly distributed that under low magnification they imparted a diffuse reddish color to the affected areas. The outer layers of the spontaneous lesions in the media showed the most abundant accumulation of lipoids. The inner layers were partly calcified, and there was only a small amount of stainable fatty material in them. The intima over the medial lesions showed the presence of numerous fine droplets of anisotropic lipoid material deposited in the swollen subendothelial ground substance, and some of the lining endothelial cells contained a few fatty particles, but there was no proliferation of cells in the intima.

In neither of these rabbits could any fatty material be observed in the neighboring normal parts of the media or intima, nor was there any deposit of lipoid elsewhere in their aortas. In other experiments of similar duration no trace of fatty deposit was seen in aortas which showed no spontaneous lesions of the media.

Typical spontaneous medial lesions of the aorta with superimposed lesions of experimental cholesterol arteriosclerosis were observed also in one of the longer experiments (that on rabbit 10) which had lasted one hundred and ten days. On gross examination of the aorta the lesions unassociated with spontaneous medial changes had an appearance and distribution similar to those seen in the other long experiments. However, in the abdominal aorta the fatty plaques in the intima were much larger than elsewhere and showed a curious configuration. Each plaque of intimal thickening was ring-shaped, with an umbilicated center, so that the lesions looked like small craters. In microscopic sections it was noted that the central depressed areas were the sites of calcified spontaneous lesions of the media. Their calcified portions showed no stainable fat, but their peripheral parts where calcification had not occurred showed dense diffuse deposits of anisotropic lipoids lying free in the ground substance of the injured media. The intimal thickenings had essentially the same structural characters as those which occurred independently of the spontaneous medial lesions, but the thickening of the

intima was always greatest opposite the peripheral parts of the medial lesions and became progressively thinner toward the centers, producing the umbilicated appearance seen in the gross specimen.

COMMENT

The earliest changes which have been described as occurring in the intima of the aorta in the present experiments correspond with those which have been observed by other investigators. As in the experiments of Anitschkow [3] and of others, the earliest deposits of lipoid material in the intima were seen to be free in the swollen subendothelial ground substance. The older discussions of the intimal lesions indicated that the first deposition of lipoids took place without the prior occurrence of any other change. Swelling of the subendothelial layer of the intima was observed at the time of the earliest appearance of the lipoids, but the change in the subendothelial layer was interpreted as the result of the presence of the lipoid deposits, and this view was maintained in the subsequent literature. Contrary to this older interpretation, it now seems probable that the swelling of the subendothelial ground substance is a change which precedes the deposition of lipoids in those areas. The microscopic appearance of the very early intimal lesions clearly suggests such a conclusion. This view is supported by a great deal of indirect evidence [1] and is consistent with the direct observations recorded here as well as with those of previous investigators.

As the intimal lipoid deposits grow larger varying numbers of cells appear in the intima, but the quantity of lipoids which can accumulate in the subendothelial layer of the intima prior to the appearance of any cells is such as to leave no doubt that deposition of lipoid can take place without the necessity of any activity on the part of phagocytic cells. The cells which participate in the intimal reaction consist of two types, macrophages and fibroblasts, but the cellular response is subject to considerable variation. Large numbers of lipoid-containing macrophages or foam cells may rapidly appear and dominate the picture in the early stages, while the intercellular ground substance and the lipoids in association with it remain inconspicuous. Such lesions seem to occur most frequently and have come to be spoken of as typical. Nevertheless, as has been found in the present experiments, foam cells and fibroblasts may be few and the intercellular material abundant. Under these circumstances the few cells contain only a very small proportion of the total lipoids in the intima, while the bulk of the fatty material is held in association with the subendothelial intercellular ground substance. There is a considerable variation also in the activity of connective tissue proliferation. Fibroblasts may apear in abundance early in the formation of

3. Anitschkow, N.: Beitr. z. path. Anat. u. z. allg. Path. **56**:379, 1913.

the lesions, or they may remain merely a few for a long time, forming only an inconspicuous part of the intimal thickening.

The development of the lesions in the media in the present experiments followed a more constant course. Small focal areas of necrosis appeared in the inner layers of the media. At first these lesions were totally free from materials stainable with sudan III, but as they grew larger they became impregnated with increasingly dense accumulations of anisotropic lipoids which lay free in the flocculent ground substance occupying the areas of muscle destruction. There were, however, wide variations in the extent of the medial lesions. Occasionally, even beneath well developed intimal plaques the media was affected only to the slightest degree, but more frequently it showed extensive lesions, especially in the arch of the aorta and in the pulmonary artery.

The observation of medial lesions such as have been described here is not a new one. Similar lesions have been encountered by other investigators, but their occurrence has been consistently minimized or completely neglected. It is not questioned that lesions in the media have been lacking in some instances. Such careful observers as McMeans and Klotz [4] observed that medial changes in the arteries in their experiments were the exception rather than the rule, but among the authors in whose experiments lesions in the media were apparently prominent only Bailey [5] has given them any consideration. Although such medial lesions have been observed by others, their occurrence prior to any intimal changes or independent of alterations in the intima over the affected areas in the media, as was noted in the present experiments, has not previously been reported.

The lesions in the media, especially those which are independent of changes in the intima, are of considerable importance in the interpretation of the significance of experimental cholesterol arteriosclerosis, since they furnish additional evidence bearing directly on the etiology and pathogenesis of the arterial lesions. Furthermore, it should be emphasized that the medial changes form an integral part of experimental cholesterol arteriosclerosis and can scarcely be neglected in an accurate comparison between the morphologic features of the latter and those of arteriosclerosis in human beings.

Concerning the pathogenesis of experimental cholesterol arteriosclerosis certain points are clear. It seems obvious and is generally agreed that the quantity of lipoid material which accumulates in the arterial lesions is too great to be derived from the breakdown of tissue at the site. Indeed, it seems probable that only a negligible part of it arises in this way. It becomes necessary, therefore, to explain the

4. McMeans, J. W., and Klotz, O.: J. M. Research **34**:41, 1916.
5. Bailey, C. H.: J. Exper. Med. **23**:69, 1916.

process by which lipoids make their way into the arterial wall and accumulate there in appreciable quantities. It is clear that the lipoid substances must be carried to their destination in the arterial wall through the intermediation of the blood plasma flowing through the vasa vasorum or through the lumen of the artery. Furthermore, the plasma or fluid derived from it must permeate the tissue spaces of the walls of the vessels to answer the nutritional requirements of the latter. That this nutritive fluid can carry with it certain colloids as well as crystalloids is capable of experimental demonstration.[6] This being so, it seems reasonable to believe that lipoids in colloidal solution in the blood plasma can also enter the arterial wall in this way. Thus the wall of the artery is viewed as being permeated by a nutritive fluid which has a certain content of lipoids. It is evident that this fluid must be the immediate source of the lipoid substances of extraneous origin which accumulate in the arterial lesions.

The ideas expressed in the preceding paragraph seem reasonable and credible, and they correspond with those involved in the "infiltration" theory as applied by Anitschkow [7] to the development of the experimental arterial lesions. Beyond this point, however, lies the difficulty of explaining the occurrence, under the conditions of the experiments, of precipitation of lipoids from the nutritive fluid so that they accumulate in certain areas in the walls of the arteries in stainable form.

It is known that the feeding of large amounts of cholesterol to rabbits results in a greatly increased content of lipoids in the blood, and presumably this increase is paralleled by a similar rise in the lipoid content of the nutritive fluid permeating the arterial wall. However, such an increased concentration of lipoids is not sufficient in itself to account for their precipitation for the reason that the deposition of lipoid materials in the arterial wall does not begin as a diffuse process but occurs only in certain limited areas whether in the intima or media. It is evident, therefore, that local conditions which are able to bring about the deposition of lipoids must exist in the walls of the arteries in the areas where lipoid deposits occur, and it is equally obvious that these conditions must exist before the precipitation of lipoids begins. This argument is supported by the observation of alterations in the walls of the vessels which evidently precede the commencement of the deposition of lipoid.

The earliest appearance of lipoids in the intima is seen microscopically as an extracellular deposit of fatty substances in the subendothelial

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6. Duff, G. L.: Am. J. Path. **8**:219, 1932.

7. Anitschkow, N.: Experimental Arteriosclerosis in Animals, in Cowdry, E. V.: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933, chap. 10.

ground substance. The subendothelial layer, even at that early stage, is definitely swollen not only in the areas where fatty deposits are noted but also in the adjacent parts of the intima where lipoid materials have not yet been deposited. The latter areas are subsequently occupied by lipoid deposits, but swelling of the subendothelial ground substance appears always to be the forerunner, and it seems entirely probable, therefore, that a similar swelling of the intima precedes the earliest deposition of fatty substances. In the case of the lipoid deposits in the media, it is clear that focal destruction of muscle fibers occurs prior to the accumulation of lipoids in the damaged areas. Thus, the development of the local conditions in the arterial walls which are responsible for the subsequent precipitation of lipoids is signified by the apearance of visible alterations which in the media at least and probably also in the intima are the result of some sort of injury. Arterial injury is the initial event and is followed by the deposition of lipoids in the damaged regions. The altered intercellular ground substance in the injured areas seems to have a special predilection for the accumulation of lipoid substances.

With these facts in view, one can expect that direct injury to the walls of the arteries of cholesterol-fed rabbits will greatly facilitate the deposition of lipoids in the damaged areas, and some experiments carried out by Ssolowjew [8] demonstrated that this is the case. By light cauterization of segments of the arteries or by mechanical injury this investigator was able to obtain deposition of lipoids in the injured areas in a much shorter time than is possible in rabbits in which the arteries are presumably normal at the commencement of cholesterol feeding. These and other experiments demonstrating the effect of arterial injury in facilitating deposition of lipoid are summarized in more detail in a review already published.[1]

In some of the present experiments (those on rabbits 7, 10 and 12) similar results were obtained. In these experiments a less artificial form of injury was provided by the chance occurrence of spontaneous medial lesions of the aorta, which, when they occurred in control animals, were free from anisotropic lipoids. In the cholesterol-fed rabbits they rapidly became impregnated with deposits of anisotropic lipoids. These deposits appeared in the intima and media in the injured areas before any other parts of the aorta were affected and in a much shorter time than was necessary to produce lesions in the otherwise normal vessels of other rabbits on identical diets. These experiments show clearly that the general conditions in the blood and in the nutritive fluid which permeates the arterial walls may be suitable for the deposition of lipoids in the

8. Ssolowjew, A.: Ztschr. f. d. ges. exper. Med. **69**:94, 1930; Virchows Arch. f. path. Anat. **283**:213, 1932.

walls of the arteries without the occurrence of the precipitation of lipoid anywhere except in injured areas. The obvious conclusion is that the injury to the walls of the arteries represented by the easily recognizable spontaneous lesions of the media was a preliminary condition indispensable to the subsequent precipitation of lipoids in those areas. In one experiment of longer duration the areas of arterial injury were marked by a much more abundant deposit of lipoids than occurred anywhere else in the aorta. All these observations are in agreement with the results of all other recorded experiments bearing on the question of the influence of injury to the arteries in the development of experimental cholesterol arteriosclerosis.

The conclusions which may be drawn from these experiments confirm those already reached on the basis of observations on the early lesions of experimental cholesterol arteriosclerosis. All these experimental observations indicate clearly that the deposition of lipoids in the walls of the arteries occurs only after the preliminary development of initial alterations representing the effect of some form of arterial injury. The lipoid deposits are really an index of injury to the walls of the vessels.

Current theories advanced to explain the development of experimental cholesterol arteriosclerosis in rabbits scarcely touch on the rôle of injury to the arteries. Interest is constantly centered on the increased content of lipoids in the blood. The hyperlipemia is doubtless of importance in the experiments on animals, but it is necessary also to recognize the importance of the primary arterial injury which leads to the subsequent deposition of lipoids.

Although it has been shown that the cholesterol content of the blood is greatly increased in rabbits by the feeding of cholesterol, it has never been demonstrated that this hypercholesterolemia is directly responsible for the development of the lesions in the arteries. It can be said that some degree of hypercholesterolemia is in all probability one essential factor in the production of experimental cholesterol arteriosclerosis in rabbits. This has never been proved conclusively, and, though probably true, it remains an assumption which is justified only by circumstantial evidence. There is no justification, however, for the further assumption that hypercholesterolemia of itself will produce the lesions in the arteries, for there is definite evidence to the contrary. All the experimental evidence bearing on the question indicates that some unrecognized factor inherent in the method of cholesterol feeding effects an injury to the walls of the arteries. At the same time the level of cholesterol in the blood, which is normally very low in rabbits, is greatly increased. The injured areas in the walls of the vessels in the presence of hypercholesterolemia become the sites of deposition of lipoid, and the charac-

teristic lesions of experimental cholesterol arteriosclerosis develop. It is evident that the local factor of arterial injury ranks equally in importance with the general factor of hyperlipemia. The experimental data on which these statements are based need not be detailed here since the whole question has been dealt with fully in a discussion of the etiology and pathogenesis of experimental cholesterol arteriosclerosis in a recent publication.[1]

The interpretation of the significance of the experimental results in relation to human arteriosclerosis is obviously of paramount importance and deserves more careful consideration than it has generally received in the past. The tendency has been to apply directly to man the results of the experiments on cholesterol feeding without regard for important differences between the rabbit and man for which certain allowances must be made. This has led to some conclusions which are misleading. For example, the inference has been drawn on the basis of the experimental data that hypercholesterolemia, occurring as the result of some disorder of the cholesterol metabolism, is to be regarded as the cause of arteriosclerosis in man. Attempts to demonstrate an elevation of the cholesterol content of the blood in association with the development of arteriosclerosis in man have failed to provide convincing evidence in support of the hypothesis, and no other evidence to indicate a disorder of lipoid metabolism has been found,[1] but some investigators maintain their original contention that a general disturbance of the cholesterol metabolism is the fundamental cause of human arteriosclerosis. The experimental results alone and unsupported do not justify this conclusion.

The differences between the experimental animal and man, added to the anatomic differences between experimental cholesterol arteriosclerosis and human arteriosclerosis, render the problem of interpretation far from simple. Indeed, when all the circumstances are taken into consideration, it becomes evident that the application of conclusions drawn from the experiments cannot be justified without some evidence of the existence of comparable conditions in the human being. A proper appreciation of the significance of the experimental results can be gained only through correlation of the experimental data with corresponding data derived from the study of human material. Such a correlation has been undertaken elsewhere,[1] and it will suffice in this paper to summarize briefly the general conclusions reached.

In the case of man, as in the rabbit, there is every reason to believe that some form of injury to the arterial walls precedes and is responsible for the deposition of lipoids in the lesions of arteriosclerosis. The lipoid deposits are an index of injury to the walls of the vessel, but the cause of the primary arterial injury is unknown. Contrary to the state of affairs in the rabbit, however, the cholesterol content of the

blood in man is normally high, its concentration being about double that found normally in the rabbit. Not only is cholesterol present in greater concentration in human plasma, but it stands normally at a level not far from the point of saturation. It is not astonishing, therefore, that the normal level of the blood cholesterol in man is sufficiently high to permit the deposition of lipoids in arteriosclerotic lesions, even though the low normal level of the blood cholesterol in the rabbit requires to be elevated before this occurs. Hypercholesterolemia is not found with any regularity in association with human arteriosclerosis, and it seems highly probable that arteriosclerosis in man can and usually does develop without deviation of the cholesterol content of the blood beyond the normal limits of variation. Hypercholesterolemia, when it occurs, may be expected on theoretical grounds to accelerate the development of arteriosclerotic changes which have already been initiated, but hypercholesterolemia of itself cannot be regarded as a cause of human arteriosclerosis. At present the primary arterial injury must be regarded not only as the initial event in the development of arteriosclerosis in man but as the one abnormal factor responsible for the subsequent train of events.

Certain criticisms which have been directed against this interpretation by Leary [9a] must be mentioned in order to correct the erroneous impression which he has conveyed regarding my attitude toward the experimental data. Since mine is the only publication specifically referred to as the object of his attack, it would seem that I am held responsible for the list of "criticisms . . . of the use of the rabbit as an experimental animal" with which he has opened the argument. It is true that the points that he has mentioned were raised in my discussion, not, however, as grounds for the rejection of the experimental results, as Leary has implied, but simply as facts which must be considered in their interpretation. I have never expressed the slightest objection to the use of the rabbit as an experimental animal, but I still insist that differences between the rabbit and man must be given due consideration in the interpretation of the significance of the experimental data as applied to the human being. The remainder of Leary's paper consists of a reiteration of the conventional arguments which for years have been used to explain away the obvious differences between experimental cholesterol arteriosclerosis and the disease in man and to support the so-called cholesterol etiology of human arteriosclerosis. These arguments have been dealt with in the review referred to and need not be repeated here. In the same paper my own position is clearly stated and requires no further defense.

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9. Leary, T.: (*a*) Arch. Path. **21**:459, 1936; (*b*) ibid. **21**:419, 1936.

It is perhaps worth noting that in spite of his criticism of my work Leary has been considerably influenced by it. In his earlier publications he maintained that an upset or insufficiency of the general cholesterol metabolism was the fundamental cause of human arteriosclerosis, but he has now relinquished this position to the extent of admitting that the disturbance of the cholesterol metabolism may amount to no more than a local derangement in the tissue exchange of this substance at the site of arteriosclerotic lesions.[9] Regarding the formation of the arterial lesions themselves, Leary formerly stated that the deposition of cholesterol in the arterial wall was the primary event. But the repeated references in his recent papers to "the initial mucoid change" in the subendothelial layer of the intima which prepares the intimal ground substance for the subsequent deposition of cholesterol [9] stand as evidence of his acceptance of my thesis that the initial stage in the development of human arteriosclerosis consists of preliminary local changes in the walls of the arteries which precede the deposition of lipoids.[1]

SUMMARY AND CONCLUSIONS

Experimental arteriosclerosis was produced in rabbits by feeding cholesterol-rich diets. The experiments extended over periods of from eight to one hundred and nineteen days.

The lesions observed in the aorta are described. Deposition of anisotropic lipoids occurred in both the intima and the media. In both situations histologic changes preceded the deposition of lipoid materials. In the media these changes were especially striking and were clearly the result of some form of injury. The lesions in the intima and media usually occurred together, but early lesions in the media were noted on occasion in the absence of any change in the overlying intima, and the reverse was also true.

The effect of injury to the aorta was studied in three experiments. Injured areas in the aortic wall became impregnated with anisotropic lipoids much more rapidly and more abundantly than other parts of the aorta. Lipoids accumulated in these areas in a much shorter time than was necessary for the production of arterial lesions in normal animals on identical diets.

It is pointed out that the experimental lesions of the arteries may vary considerably in their histologic appearance from the so-called typical picture. The fact is emphasized that the lesions in the media form an integral part of this experimental disease of the arteries.

In a consideration of the pathogenesis of experimental cholesterol arteriosclerosis in rabbits special emphasis is laid on the rôle of injury to the walls of the arteries. The importance of hyperlipemia in the experiments on animals is recognized, but it is concluded on the basis of

the experimental observations that the deposition of lipoids in the walls of the arteries takes place only after the occurrence of a primary injury to the walls of the vessels.

The significance of the experimental results in relation to the etiology and pathogenesis of human arteriosclerosis is touched on briefly. This subject has been discussed in detail in another publication.[1]

THROMBOSIS OF THE AORTA AND CORONARY ARTERIES

WITH SPECIAL REFERENCE TO THE "FIBRINOID" LESIONS

EUGENE CLARK, M.D.

IRVING GRAEF, M.D.

AND

HERBERT CHASIS, M.D.

NEW YORK

"FIBRINOID" LESIONS OF ATHEROSCLEROSIS AND SYPHILITIC AORTITIS AND THEIR RELATIONSHIP TO THROMBUS FORMATION IN THE AORTA

Since its introduction by Neumann [1] in 1880, the term fibrinoid has been used to designate a substance encountered in the walls of affected arteries and elsewhere which in its tinctorial behavior more or less resembles fibrin. Our interest in this lesion was stimulated by the frequent observation of areas staining like fibrin in the plaques of atherosclerosis and syphilitic aortitis on which bland thrombi had formed.

Mallory,[2] in 1912, described fibrin-like material in the intimal plaques of atherosclerotic and syphilitic aortitis, and he expressed the belief that this substance represents organizing fibrin. However, several reports have appeared recently in which the fibrin-staining, or "fibrinoid," material in such intimal plaques has been interpreted as representing degenerated or necrotic fibrous tissue, a view previously expressed by Neumann.[1] The subject has gained new and wider importance by the assertions of Jaeger [3] and of Leary [4] that thrombosis may be brought about when "fibrinoid necrosis" extends to the surface of the plaque.

Jaeger's [3] interpretation was based on the observations on the intimal plaques in four fatal cases of thrombo-angiitis obliterans and in two cases of two thrombosed atherosclerotic vessels and on a platelet thrombus deposited on an intimal plaque of syphilitic aortitis. He described the

From the Department of Pathology, Bellevue Hospital, and the Department of Pathology of New York University College of Medicine.

1. Neumann, E.: Arch. f. mikr. Anat. **18**:130-150, 1880; Virchows Arch. f. path. Anat. **144**:201-238, 1896.

2. Mallory, F. B.: The Infectious Lesions of Blood Vessels, Harvey Lectures, 1912-1913, Philadelphia, J. B. Lippincott Company, 1913, p. 150; The Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1929, p. 438.

3. Jaeger, E.: Virchows Arch. f. path. Anat. **284**:526-583 and 584-622, 1932.

4. Leary, T.: Arch. Path. **17**:453-493, 1934.

occurrence on the surface of intimal plaques of 'fibrinoid" masses fully comparable to those seen within the plaque, yet he considered the surface plaques as due to a deposit and the inner plaques as derived from collagenous tissue by necrosis. The demonstration of argyrophilic fibrils within the "fibrinoid" masses in the intimal plaques led him to consider these masses as constituting an intrinsic change within the plaque.

Leary [4] noted "regions of fibrinous or fibrinoid necrosis" in the fibrous tissue of intimal plaques in six thrombosed coronary arteries. He suggested that thrombosis occurred when the necrosis reached the endothelial layer. The necrosis was interpreted as being the result of a diminution in nutrition occurring with progressive growth of the plaque.

Jucker [5] recently reported a study of necrosis in arteriosclerotic plaques. He recognized the occurrence of material with tinctorial and morphologic properties similar to those of fibrin within the fibrous regions of intimal plaques. He expressed the opinion that in some instances this material represents necrotic or degenerated collagenous fibers on which infiltrated fibrinogen has been precipitated; in other lesions changes were observed which suggested that loosening of the bundles of connective tissue permitted penetration of plasma, which underwent coagulation.

We reached the conclusion that in the study and evaluation of the "fibrinoid" lesions in thrombosed arteries it would be of aid to examine the intimal plaques of atherosclerosis and syphilitic aortitis in nonthrombosed vessels, for in such plaques the earlier stages in the development of this lesion should be seen. The observations were compared with those made in a study of nine parietal aortic thrombi.

MATERIAL AND METHODS

Blocks were cut from forty atherosclerotic aortas presenting raised intimal plaques, ulcerated or nonulcerated, without visible thrombi. Comparable blocks were cut from thirty-eight unselected specimens exhibiting evidence of syphilitic aortitis, consisting of medial scarring, necrosis or vascularization, together with infiltration with perivascular mononuclear cells and endarteritis of the vasa vasorum. In each instance an effort was made to choose the thickest plaques available, and from two to ten blocks were selected from each specimen.

For the study of the intimal lesion of aortic thrombosis, nine specimens exhibiting this lesion were employed, two of which presented evidence of syphilitic aortitis.

The material was generally fixed in solution of formaldehyde; less frequently material preserved in Kaiserling's solution was used. Paraffin embedding was uniformly employed. Successive sections from each block were stained with Delafield's hematoxylin and eosin stain, with the Weigert elastic tissue stain combined with the Van Gieson stain, and with the Mallory phosphotungstic acid and hema-

5. Jucker, P.: Virchows Arch. f. path. Anat. **295**:301-333, 1935.

toxylin stain. In instances in which "fibrinoid" areas appeared sections were stained also by the Gram-Weigert method, by the silver impregnation method of Foot and Foot with counterstaining with the rosaniline salt of trinitrophenol and with the benzidine (Lepehne) stain for hemoglobin. The benzidine stain was employed because it was recognized that the tinctorial behavior of red blood cells and hemoglobin was similar to that of the "fibrinoid" masses when eosin, rosaniline picrate or phosphotungstic acid and hematoxylin were used.

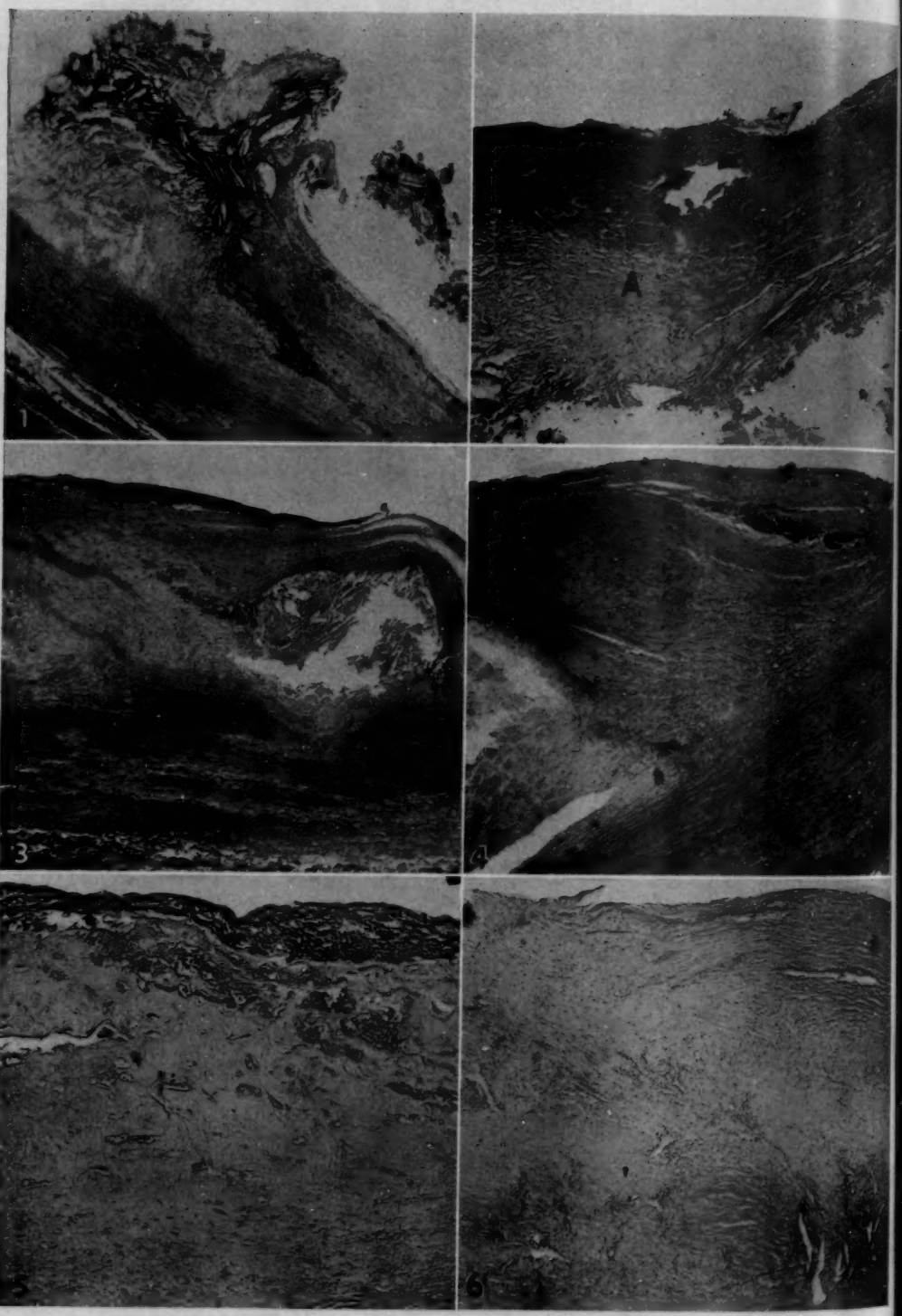
ATHEROSCLEROSIS AND SYPHILITIC AORTITIS WITHOUT GROSSLY VISIBLE THROMBOSIS

In the description which follows the term "fibrinoid" will be used, for convenience, in designation of that material which occurs either within the intimal plaque or as a homogeneous membrane on its surface and which in its staining properties is similar to fibrin. Though the reaction of this material to gentian violet was not constant, a similar inconstancy was observed in the fibrinous component of thrombi. With the other stains employed no essential differences were discerned between the "fibrinoid" material in the plaque and the fibrinous component of thrombi, except for the presence of argyrophilic fibrils within or surrounding the "fibrinoid" material in the plaque.

In the study of these plaques we separated the lesions in which the fibrin-staining material occurred in the lipoid zones of the plaques from the lesions characterized by its occurrence in the fibrous regions. The genesis of each of these two lesions is, in most instances, decidedly different. The lesions in which the fibrin-staining material was present in the fibrinous regions were of chief interest because it has been asserted that they play an important rôle in the causation of thrombosis.

In twenty-two of the forty atherosclerotic aortas studied ulcerated plaques were sectioned. In seventeen of these aortas fibrin-staining material was present within the lipoid zones of some ulcerated plaques. Somewhat less often fibrin-staining material was observed in the lipoid zones of sections through intact plaques (twelve of thirty-seven cases). The fibrin-staining material presented a fine, thready or coarsely fibrillar appearance and occupied the intervals between the acicular spaces (fig. 1). Elsewhere it appeared as refractile pools of homogeneous substance or presented a dull, smudgy appearance mixed with fatty detritus. In one instance the smudgy material showed a positive reaction to benzidine, but a negative result was observed in all others. Leukocytes and red blood cells were infrequently present.

"Fibrinoid" masses were noted either on the surface or within the superficial fibrous regions of the plaques in sections from fifteen of the forty atherosclerotic aortas. They were observed in intact plaques almost as frequently as in ulcerated ones (fig. 2) and were present as commonly in fibrous intimal plaques possessing little lipoid material as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EXPLANATION OF FIGURES 1-6

Fig. 1.—Sections through an ulcerated atherosclerotic plaque of an aorta, exhibiting "fibrinoid" material between the acicular spaces of the atheromatous portion and in the fibrous regions of the plaque. The "fibrinoid" material appears black. Phosphotungstic acid and hematoxylin stain.

Fig. 2.—Sections through an ulcerated atherosclerotic plaque of an aorta, exhibiting a broad layer of "fibrinoid" material. Note the extensions of the "fibrinoid" material (appearing dark gray) into the fibrous covering of the atheromas (*A*). Hematoxylin and eosin stain.

Fig. 3.—Sections through an intact atherosclerotic plaque of an aorta, revealing the presence of "fibrinoid" material on the surface of the fibrous covering of the atheroma. There are traces of material staining similarly in the atheromatous portion as well. Phosphotungstic acid and hematoxylin stain.

Fig. 4.—Sections through an intact atherosclerotic plaque of an aorta, showing subsurface "fibrinoid" masses in the superficial fibrous region adjacent to the atheromatous portion. Phosphotungstic acid and hematoxylin stain.

Fig. 5.—Sections through an intact fibrous plaque of an aorta. Note the absence of atheromas and the presence of surface and subsurface "fibrinoid" masses. Many small blood channels are visible in the cellular plaque. Hematoxylin and eosin stain.

Fig. 6.—Sections through a plaque of syphilitic aortitis, revealing the presence of subsurface "fibrinoid" masses in the fibrous intimal plaque. The medial scarring and cellular infiltration are prominent. Hematoxylin and eosin stain.

in the fibrous covering of atheromas. In a few instances the fibrin-staining masses appeared only as a membrane on the surface of the plaque (fig. 3), and in others the material appeared only in the superficial regions of the plaque covered by endothelium or connective tissue (subsurface; fig. 4). But in the majority of instances these fibrin-staining masses were both surface and subsurface in position (fig. 5).

In seventeen of the thirty-eight aortas exhibiting syphilitic aortitis, comparable fibrin-staining masses were observed on the surface and within the superficial fibrous regions of the intimal plaques (figs. 6 to 8). In eleven of these the "fibrinoid" material occurred in hyaline plaques which appeared free from lipoid material or calcium deposits.

The "fibrinoid" or fibrin-staining material was readily identified by its homogeneous and refractile character and by its tinctorial properties. It was most commonly seen in the form of narrow bands (0.015 to 0.15 mm.) lying on the surface of the plaque and continuous with similar masses which extended obliquely or horizontally in the underlying fibrous tissue. In other instances ulcerated bands of "fibrinoid" material were seen running horizontally in the plaque, parallel with the connective tissue fibers with which they dovetailed. At times the "fibrinoid" bands formed multiple horizontal layers. Occasionally "fibrinoid" material was present more deeply in the interstices of the collagenous fibers of the plaque, appearing to penetrate from an ulcerated atheroma (fig. 1).

The character of the fibrous tissue of different plaques was variable, at times appearing dense, with closely packed parallel fasciculi, and in other instances presenting a looser texture, with widely separated collagenous fibers. In the majority of instances plump fibroblasts were seen in the connective tissue contiguous with the "fibrinoid" masses. In the same section some "fibrinoid" areas were encompassed by fibrous tissue with many young fibroblasts, whereas elsewhere such fibroblasts were lacking around similar masses. Capillary formation in the fibrous zones of intimal plaques surrounding the "fibrinoid" bands was observed in only one instance (fig. 5).

Argyrophilic fibrils were almost constantly present in or about the "fibrinoid" masses. In many instances the black fibrils appeared to form a rim in contact with and surrounding the "fibrinoid" mass but not actually lying within the latter. In others, however, they clearly lay within the mass, appearing as separated linear structures somewhat parallel to the collagenous fibers in which they terminated (fig. 9).

The fibrin-staining masses uniformly yielded a negative benzidine reaction.

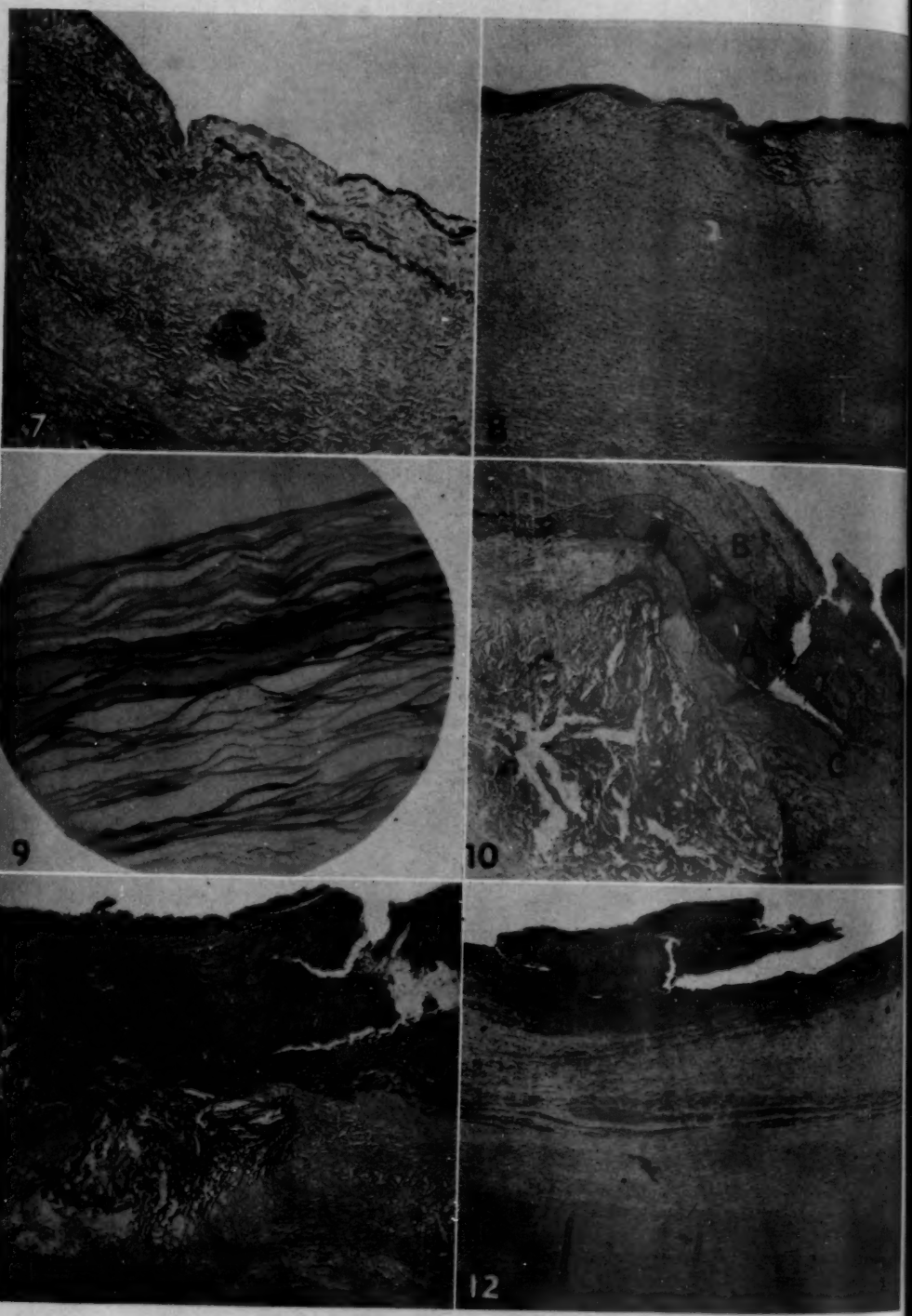
Comment.—What is the nature of the "fibrinoid" material? The common localization of fibrin-staining masses on the surface of the

intimal plaques and their limitation to the superficial regions of these plaques at once suggest that these masses represent material which is derived from the blood stream. A gradual transition from masses which are clearly surface deposits to those covered by endothelium and collagenous tissue containing proliferating fibroblasts may be observed in the sections. This yields strong support for the interpretation that the subsurface "fibrinoid" masses represent the remnants of a partially organized surface deposit. The appearance in some plaques of multiple layers of homogeneous fibrin-staining material separated by fibrous tissue suggests that the deposition of blood elements may occur repeatedly on the same site and before the organization of the initial or subsequent deposits is completed. Such pictures are in agreement with Mallory's view that organization of deposited fibrin leads to increased growth of the plaques of syphilitic aortitis and of the fibrous component of the plaques of atherosclerosis. Our experience showing that this replacement of fibrin is performed entirely by fibroblasts also coincides with Mallory's descriptions. In only one instance did we encounter vascularization of the fibrous tissue in the region of the fibrinoid deposits.

Penetration of a plaque by blood elements may bring about also the deposition of homogeneous fibrin-staining masses within its interstices. It is common to meet macroscopic evidence of such penetration in ulcerated aortic plaques which present purplish-red or greenish intimal zones. Such instances of frank hemorrhage into the plaque have been omitted from the series reported. Wherever such plaques were examined, homogeneous "fibrinoid" masses could be seen between collagenous fibers. It is readily conceivable that some plaques may afford the opportunity for penetration and dissection by blood plasma but not for the influx of cellular elements.

There is nothing to suggest that these masses represent necrotic collagenous fibers. Evidence of an exudative cellular response has never been observed in such foci, and well preserved fibers staining as collagen are seen side by side and even dovetailing with fibrin-staining bands. Moreover, the uniform localization of these "fibrinoid" bands to the superficial portions of the plaques weakens the belief that the bands represent a degenerative change on the basis of locally diminishing nutrition.

The demonstration of argyrophilic fibrils in the "fibrinoid" lesions yields no proof that these lesions represent degenerated or necrotic collagenous fibers. In many instances the fibrils impregnated with silver have probably been laid down by fibroblasts in the process of organization (see page 195). In other instances, if organization can be excluded, the appearance of the argyrophilic structures may be due to the splitting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Explanation of Figures 7-12

Fig. 7.—Section through a plaque of syphilitic aortitis, exhibiting surface and subsurface horizontal bands of "fibrinoid" material. Note the cellular character of the plaque and the absence of vascularization. Phosphotungstic acid and hematoxylin stain.

Fig. 8.—Section of a plaque of syphilitic aortitis. Note the surface band of homogeneous "fibrinoid" material and the cellular nature of the underlying tissue. Hematoxylin and eosin stain.

Fig. 9.—Drawing of a section through an intact fibrous plaque. The section was impregnated with silver (Foot and Foot) and counterstained with Van Gieson's stain. Note the homogeneous "fibrinoid" areas partly outlined and partly traversed by roughly parallel argyrophilic fibrils, which appear black in the drawing.

Fig. 10.—Aortic thrombus in case 1. Section through a parietal thrombus exhibiting a broad layer of homogeneous "fibrinoid" material (*A*) between the formed elements of the thrombus (*B*) and the fibrous covering of the atheroma. Note the presence of a narrow band of "fibrinoid" material within the superficial fibrous region of the plaque (*C*). Hematoxylin and eosin stain.

Fig. 11.—Aortic thrombus in case 1. Another section through the same thrombus shown in figure 10, revealing the presence of a "fibrinoid" layer between the formed elements of the thrombus and the plaque. Note the gradual transition from the homogeneous "fibrinoid" layer to the formed elements of the thrombus and the continuity with "fibrinoid" material in the lipoid and fibrous zones of the plaque. Phosphotungstic acid and hematoxylin stain.

Fig. 12.—Aortic thrombus in case 2. A section through a parietal thrombus in which a fresh deposit of recognizable blood elements has been superimposed on a broad homogeneous "fibrinoid" layer which dovetails between the collagenous fibers of the plaque. Note the continuity with the oblique and horizontal bands of homogeneous "fibrinoid" material in the deeper portions of this fibrous plaque. Gram-Weigert stain.

of the collagenous fiber into its component fibrils (Mallory and Parker[6]) either before or after penetration and microscopic dissection of the plaque by blood elements.

The tinctorial behavior of the fibrin-like masses, except for the presence of argyrophilic fibrils, is in no way dissimilar from that of fibrin. Aschoff[7] stated that blood platelets may rapidly condense so as to form hyaline masses yielding a fibrin reaction. We have not been able to identify platelets as such in the "fibrinoid" lesions, though they may readily enter into the composition of the mass, and we have found no evidence to suggest that their presence is responsible for the tinctorial behavior observed. These masses have yielded a negative reaction to the benzidine stain for hemoglobin, though it must be granted that the limitations of the method do not entirely exclude the presence of hemoglobin. Since the possibility of the occurrence of blood elements other than fibrin in these hyalinized bands cannot be eliminated, we have retained the term "fibrinoid."

In ulcerated plaques the manner of entrance of blood elements into the lipoid regions appears obvious, but the appearance of similar material without cellular elements in intact plaques is not so clear. However, serial sections were not made, and possibly there was microscopic fracture of the collagenous covering layer. It may also be that rarefaction of the covering layer of an expanding atheroma may permit the penetration of blood plasma with fibrinogen yet may bar the entrance of formed blood elements.

THE INTIMAL LESION IN AORTIC THROMBOSIS

The observations made in this group, comprising nine bland aortic thrombi, are summarized in table 1.

In all but one instance (case 7) a well defined layer of more or less homogeneous material which behaved tinctorially like fibrin was encountered between the plaque and the formed elements of the thrombus (figs. 10 to 13). In five instances identical material was present also in the underlying fibrous plaque. The "fibrinoid" layer appeared in most of the sections through the thrombi and occasionally extended horizontally as a surface or subsurface layer along the plaque, beyond the area possessing a visible deposit of formed blood elements.

The layer varied roughly from 0.1 to 0.4 mm. in thickness. It frequently merged gradually with the platelets, fibrin threads and cellular elements of the thrombus. Occasionally slender collagenous fibers or large elongated nuclei intervened between them. Similarly, though the

6. Mallory, F. B., and Parker, F.: Am. J. Path. 3:515-525, 1927.

7. Aschoff, L.: Verhandl. d. deutsch. Gesellsch. f. Kreislaufforsch., 1934, pp. 11-30.

TABLE 1.—*Aortic Thrombi*

Cases	Age, Years	Sex	Major Necropsy Observations	Type of Thrombus	Size of Thrombus, Cm.		Intimal Lesion Underlying the Thrombus	Location of "Fibrinoid" Material	Argyrophilic Fibrils in "Fibrinoid" Layer
					Diameters	Thickness			
1	42	M	Thrombosis of anterior descending branch of left and of right coronary artery; myocardial infarct; lobular pneumonia	Single	2 by 1	0.3	Ulcerated atherosclerotic plaques	Between thrombus and plaque; in fibrous zone of plaque; in ulcerated atheroma	Scanty
2	63	M	Carcinoma of esophagus; lobular pneumonia	Multiple	1 by 1	0.3	Thick fibrous plaques with small lipoid deposits	Between thrombus and plaque; in fibrous zone of plaque	Abundant
3	34	M	Thrombosis of left coronary artery; myocardial infarct; lobular pneumonia; syphilitic aortitis	Multiple	2 by 2	0.4	Fibrous plaques of syphilitic aortitis	Between thrombus and plaque; in fibrous zone of plaque	Abundant
4	65	M	Pemphigus; hypertrophy of heart; severe sclerosis of coronary arteries	Multiple	A few mm.	A few mm.	Ulcerated atherosclerotic plaques	Between thrombus and plaque; in ulcerated atheroma	Absent
5	59	M	Hypertrophy of heart; sclerosis of coronary arteries; mural thrombus of left ventricle; mesenteric thrombus or embolism; peritonitis	Single	1 by 1	0.5	Ulcerated atherosclerotic plaques	Between thrombus and plaque	Abundant
6	72	M	Carcinoma of esophagus; generalized peritonitis	Single, occlusive	8 by 8	...	Ulcerated atherosclerotic plaques	Between thrombus and plaque; in ulcerated atheroma	Scanty
7	49	F	Acute suppurative pyelonephrit's; subacute cystitis; gangrene of right hand and left foot	Single	1.5 by 1.5	1.0	Atherosclerotic plaque with rarefaction of fibrous covering	No "fibrinoid" layer; "fibrinoid" material in atheroma	
8	85	F	Arteriosclerosis cerebri; lobular pneumonia; thrombosis or embolism of external iliac and left hypogastric arteries	Single	1.5 by 1.5	0.5	Ulcerated atherosclerotic plaques	Between thrombus and plaque; in fibrous zone of plaque; in ulcerated atheroma	Abundant
9	50	M	Carcinoma of rectum; abscess of prostate; generalized peritonitis; syphilitic aortitis	Multiple	A few mm.	A few mm.	Fibrous and calcified plaques of syphilitic aortitis	Between thrombus and plaque; in fibrous zone of plaque	Abundant

"fibrinoid" layer contrasted sharply with the fibrous tissue of the underlying plaque, in some instances bands of "fibrinoid" material could be followed into the plaque, dovetailing with collagenous fibers. Where the "fibrinoid" layer bridged over an atheromatous ulcer and separated the atheroma from the formed thrombotic elements, it merged with homogeneous and fibrillar material of similar staining properties, accompanied by red cells and leukocytes, occupying the zones between the acicular spaces (fig. 11).

In five cases argyrophilic fibrils were abundantly present within the "fibrinoid" layer; in others they were scanty or absent. They presented an appearance similar to that which we have described in the "fibrinoid" regions of plaques without grossly visible thrombosis. Collagenous fibers were generally represented only by a few slender scattered structures, sometimes isolated in the "fibrinoid" mass, sometimes continuous with thinner silver-impregnated fibrils. Where argyrophilic fibers were abundant, plump fibroblasts could frequently be identified, but they were rarely present in large numbers and sometimes were lacking. In no instance did the "fibrinoid" layer or the plaque related to it exhibit vascularization.

Usually the "fibrinoid" layer as well as the subsurface "fibrinoid" masses yielded a negative reaction to benzidine. Occasionally a faint brownish tinge was observed in the "fibrinoid" substance when in contact with masses of erythrocytes.

Case 7 is worthy of detailed report because it convincingly illustrates the character of the organization which may be undergone by surface deposits on aortic plaques directly exposed to the force of the blood stream.

Case 7.—A woman aged 49 was transferred to the Bellevue Hospital from another institution, to which she had been admitted three weeks previously in a semistuporous state. There was a history of chronic alcoholism, for which she had been repeatedly hospitalized. Her condition exhibited a progressively downhill course characterized by stupor, fixation of the pupils and cyanosis of the face and upper extremities. Death occurred nine days after the patient's admission to the hospital. There were signs of peripheral gangrene of the left foot and right hand.

Postmortem examination revealed a mural thrombus of the ascending aorta, mild atherosclerosis of the aorta, subacute cystitis and acute suppurative pyelonephritis. Because of limitations of the autopsy the vessels of the gangrenous extremities were not examined.

The thrombus in the ascending aorta was roughly triangular, the apex facing proximally and the base almost blocking the orifice of the right innominate artery. It measured approximately 2 cm. in length and 1.5 cm. in depth. The proximal end was firmly attached to the aortic wall, but the distal end was unattached. The thrombosed area was divided into three blocks and was sectioned serially at intervals of 10 microns.

The sections showed that the thrombus was attached to an oval atheromatous plaque about 1.5 cm. in diameter, which in sections appeared crescentic. It had

a depth of 2 mm. The attachment of the thrombus was limited to the plaque. Distally it extended beyond the plaque, overhanging but unattached to the normal wall of the vessel (fig. 14). It was comprised of columns of platelets, fibrin threads, hyalinized fibrin staining masses, pyknotic leukocytes and red blood cells.

The fibrous tissue covering of the atheroma diminished progressively toward the summit of the plaque. At the top the atheroma was separated from the thrombus by only a few widely separated collagenous fibers. In the interstices of these fibers were blood cells and fibrillar fibrin. Homogeneous and fibrillar fibrin-staining material was noted in the lipoid zones of the plaque.

In the distal aspect of the plaque, i. e., facing the innominate artery, the thrombus was thick, and its basal portion was infiltrated by numerous young fibroblasts, capillaries, argyrophilic reticulum and collagenous fibers (fig. 15). However, at its proximal aspect, i. e., facing the aortic valves, the organization presented a different configuration. There the organized portion of the thrombus nakedly faced the lumen. It presented an appearance of dense homogeneous fibrin-staining masses and red blood cells. Coarse, dense, horizontal, parallel and anastomosing argyrophilic fibrils, a few collagenous fibers and a moderate number of young fibroblasts were present, but no vascularization was evident (fig. 16).

Comment.—It thus appears that the "fibrinoid" layer on which the formed elements of parietal aortic thrombi are deposited represents an earlier surface deposit of blood elements. The variability in the presence and in the abundance of the argyrophilic fibrils in the "fibrinoid" layer is in most instances a reflection of the difference in the degree of organization of the surface deposit; in others it may represent the difference in the degree of loosening and separation of the collagen fibers, either preceding or following penetration and dissection of the plaque by blood elements.

What is the relationship of the "fibrinoid" layer to thrombosis? If by thrombosis is meant the deposition of blood elements on the wall of the vessel, then the thrombotic nature of the fibrinoid layer itself becomes self-evident. The gradual transition observed in the plaques of atherosclerotic and syphilitic aortas from a thin fibrin-staining surface membrane to a broader layer of condensed homogeneous blood elements, on which finally a fresh deposit of recognizable platelet masses, blood cells and fibrillar fibrin is seen, leads one to suspect that the development of large parietal thrombi in the aorta frequently follows such a sequence. That the development of the thrombus beyond the stage of laminated and fused blood elements occurs only infrequently may be a reflection of the unfavorable circumstances afforded by a widely patent artery with a swiftly moving blood stream.

It appears that the "fibrinoid" layer largely represents laminated fibrin, though the inclusion of other blood elements which have lost their identifying features is likely. Whether clumping and adherence of platelets to the vessel wall necessarily precede the deposition of fibrin cannot be determined from the examination of the broad "fibrinoid" layers in which transformation and condensation of the elements have


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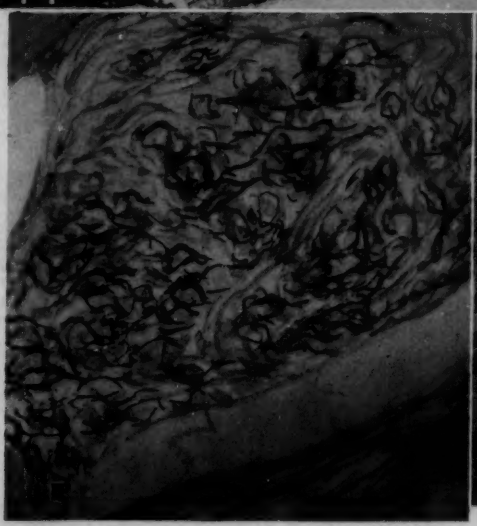


EXPLANATION OF FIGURES 13-16

Fig. 13.—A section through the thrombus and the wall of the aorta in case 8. A broad homogeneous "fibrinoid" layer (older thrombus) with subsurface extensions into the fibrous covering of the ulcerated atheroma is visible beneath the fresh thrombus. Phosphotungstic acid and hematoxylin stain.

Fig. 14.—A section through the thrombus and underlying wall of aorta in case 7. The proximal sloping aspect of the thrombus is on the right side of the field (*B*), and the distal aspect of the thrombus is on the left side (*A*). Note that the thrombus is adherent only to the plaque and that the distal portion overhangs but remains unattached to the vessel wall beyond the plaque. Hematoxylin and eosin stain.

Fig. 15.—Aortic thrombus in case 7. Drawing illustrating the character of the organization at the distal aspect of the plaque. The field corresponds to the encircled area (*A*) in figure 14. The section was impregnated with silver and counterstained with the Van Gieson stain. Note the argyrophilic reticulum (black fibers).

Fig. 16.—Aortic thrombus in case 7. Drawing prepared from the same section as that shown in figure 15, illustrating the lamellated horizontal and anastomosing argyrophilic fibrils coursing through the organizing thrombus on the proximal aspect of the plaque. The field corresponds to the encircled area (*B*) in figure 14.

most likely occurred. But in other instances, in which there is a thin homogeneous fibrin-staining membrane, the suggestion that the deposition of fibrin may occur initially seems tenable. Klemensiewicz [8] and Dietrich [9] have expressed the belief that the deposition of a "homogeneous fibrin membrane" precedes the clumping and adherence of blood platelets.

But others (Welch,[10] Aschoff [7] and Apitz [11]) have emphasized that the formation of fibrin does not occur in the first stage of thrombosis and that thrombosis is initiated by clumping and adherence of platelets to the vessel wall. It must be noted, however, that the quoted reports have been based on observations more or less limited to spontaneous or experimentally induced thrombi in veins. Whether such evidence may be applied to parietal thrombi occurring on aortic plaques is uncertain.

THE INTIMAL LESION OF CORONARY THROMBOSIS

Though it is generally considered that bland thrombosis of the coronary artery usually represents an incident in the course of coronary arteriosclerosis (Welch,[10] Jores,[12] and Benson [13]), little attention has been directed to the anatomic details which might determine thrombus formation in the involved vessel. It occurred to us that examination of serial sections of the entire thrombosed portion of the vessel might shed light on the nature of the intimal changes at the site of initial thrombosis. The report which follows embraces the study of eleven thrombosed coronary arteries which were chosen because they presented fresh or incompletely organized bland thrombi. Older, completely canalized thrombi were not included.

OBSERVATIONS ON THROMBOSED ATHEROSCLEROTIC CORONARY ARTERIES

Method of Study.—The thrombosed region of the vessel and a small zone proximal and distal to it were divided into blocks varying from 3 to 6 mm. in thickness. Zenker's fluid was used for fixation in two cases and solution of formaldehyde in the others. The blocks were embedded in paraffin, and serial sections were made every 5 microns. The longest interval between successive stained sections is recorded in table 2. Successive sections were stained with Delafield's hematoxylin and eosin stain, Weigert's elastic tissue stain combined with the Van Gieson stain, and the

8. Klemensiewicz, R.: Beitr. z. path. Anat. u. z. allg. Path. **63**:321-411, 1916.

9. Dietrich, A.: Thrombose: Ihre Grundlagen und ihre Bedeutung, Berlin, Julius Springer, 1932, pp. 1-102; Verhandl. d. deutsch. Gesellsch. f. Kreislaufforsch., 1934, pp. 48-52.

10. Welch, W. H.: Thrombosis, in Papers and Addresses, Baltimore, Johns Hopkins Press, 1920, vol. 1, pp. 110-192.

11. Apitz, K.: Zentralbl. f. allg. Path. u. path. Anat. **50**:9-16, 1930.

12. Jores, L., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 2.

13. Benson, R. L.: Arch. Path. **2**:876, 1926.

TABLE 2.—*Thrombosed Atherosclerotic Coronary Arteries*

Case	Age, Years	Sex	Major Necropsy Observations	Thrombus	Character of Plaque Underlying the Thrombus	Serial Sections, Microns*
1	53	F	Thrombosis of posterior descending branch of right coronary artery; myocardial infarct	Unorganized	Ulcerated atheroma	250
2	37	M	Thrombosis of anterior descending branch of left coronary artery; myocardial infarct	Unorganized	Ulcerated atheroma	100
3	..	M	Thrombosis of anterior descending branch of left coronary artery; myocardial infarct	Slight organization	Ulcerated atheroma	5
4	75	F	Hypertrophy of heart; thrombosis of right coronary artery; advanced arteriolar nephrosclerosis; lobular pneumonia	Advanced organization	Fibrous and lipoid plaques altered by organization	500
5	45	F	Hypertrophy of heart; thrombosis of anterior descending branch of left coronary artery; myocardial infarct; thrombosis of right auricular appendage; arteriolar nephrosclerosis	Unorganized	Atheroma with intact fibrous covering	500
6	60	F	Hypertrophy of heart; thrombosis of left circumflex artery; myocardial infarct; arteriolar nephrosclerosis	Unorganized	Atheroma with intact fibrous covering	125
7	60	M	Hypertrophy of heart; thrombosis of posterior descending branch of right coronary artery; myocardial infarct; thrombosis of right auricular appendage; lobular pneumonia; arteriolar nephrosclerosis	Unorganized	Slight intimal sclerosis	5
8	75	M	Hypertrophy of heart; thrombosis of left circumflex artery; myocardial infarct; thrombosis of pulmonary artery; lobular pneumonia	Unorganized	Atheroma with rarefied fibrous covering	15
9	61	M	Hypertrophy of heart; thrombosis of anterior descending branch of left coronary artery; myocardial infarct; organized pneumonia; arteriolar nephrosclerosis	Slight organization	Atheroma with rarefied fibrous covering	15
10	56	M	Thrombosis of anterior descending branch of left coronary artery; myocardial infarct; acute meningococcic meningitis	Advanced organization	Fibrous and lipoid plaques altered by organization	15
11	48	M	Thrombosis of anterior descending branch of left coronary artery; miliary myocardial infarct and mural thrombus	Fresh thrombus on organizing deposits of fibrin	Fibrous and lipoid plaques containing organizing fibrin deposits	5

* The figures record the longest interval between successive stained sections.

Mallory phosphotungstic acid and hematoxylin stain. Selected sections in each case were stained by the Gram-Weigert method, by the silver impregnation method of Foot and Foot, with counterstaining with the rosaniline salt of tri-nitrophenol and by the benzidine stain for hemoglobin (Lepehne).

Attention was particularly directed to the intimal change in the area of the initial thrombosis. It was assumed that in a fresh thrombus the initial site is represented by that region of the thrombus in which platelets are most prominent or appear to be most compact. In older thrombi the region exhibiting the most advanced degree of fibrin condensation, hyalinization and organization was judged to be the site of the initial thrombosis.

In nine cases the thrombi were sufficiently recent to permit recognition of the character of the intimal base on which the deposition of blood elements occurred. The following grouping is based on the nature of the intimal changes in the oldest region of the thrombus.

Group A.—In three cases (cases 1 to 3) a break in the inner fibrous lining of the atheroma was seen underlying the initially formed region of the thrombus. Through this defect in the inner fibrous lining of the plaque, fibrin-staining material of a fibrillar or homogeneous nature was continuous with material with identical staining properties in the thrombus (fig. 17). The fibrinous material outlined the acicular spaces of the atheroma and was sometimes accompanied by red blood cells and leukocytes. In cases 1 and 2 the patient was ambulatory, symptoms of coronary thrombosis appeared suddenly and death occurred within twenty-four hours. The history in case 3 is unknown; the patient was a man who was found dead.[14]

Illustrative of this group is the following protocol.

CASE 2.—Postmortem examination revealed severe coronary atherosclerosis, with diminution of the lumen of the right coronary artery and thrombosis of the anterior descending branch of the left coronary artery, and fresh infarction of the interventricular septum.

The thrombus was about 10 mm. in length, with its proximal end about 10 mm. below the inception of the affected vessel. Serial sections of the thrombosed portion were made at intervals of 100 microns. The most proximal part of the vessel exhibited concentric fibrous intimal thickening with moderate reduction of the lumen. Part of it consisted of an eccentric plaque containing lipoid material and calcium, which was separated from the lumen by only a narrow rim of a few collagenous fibers. The thrombus, incompletely filling the lumen, was composed of red and white blood cells, a conspicuous network of fibrin and a few masses of platelets. Distally, the lipoid plaque soon approached the endothelium, with which it finally came in contact, forming an eccentric subendothelial atheroma extending half-way around the lumen. Here the lumen was more nearly filled by the thrombus, which still consisted of cells and whorls of shreddy fibrin, without visible masses of platelets. Fibrillar fibrin was encountered between the lipoid cells in the plaque directly beneath the endothelium. About 300 microns distal to this

14. The Medical Examiner's Office has permitted us to report on this case.

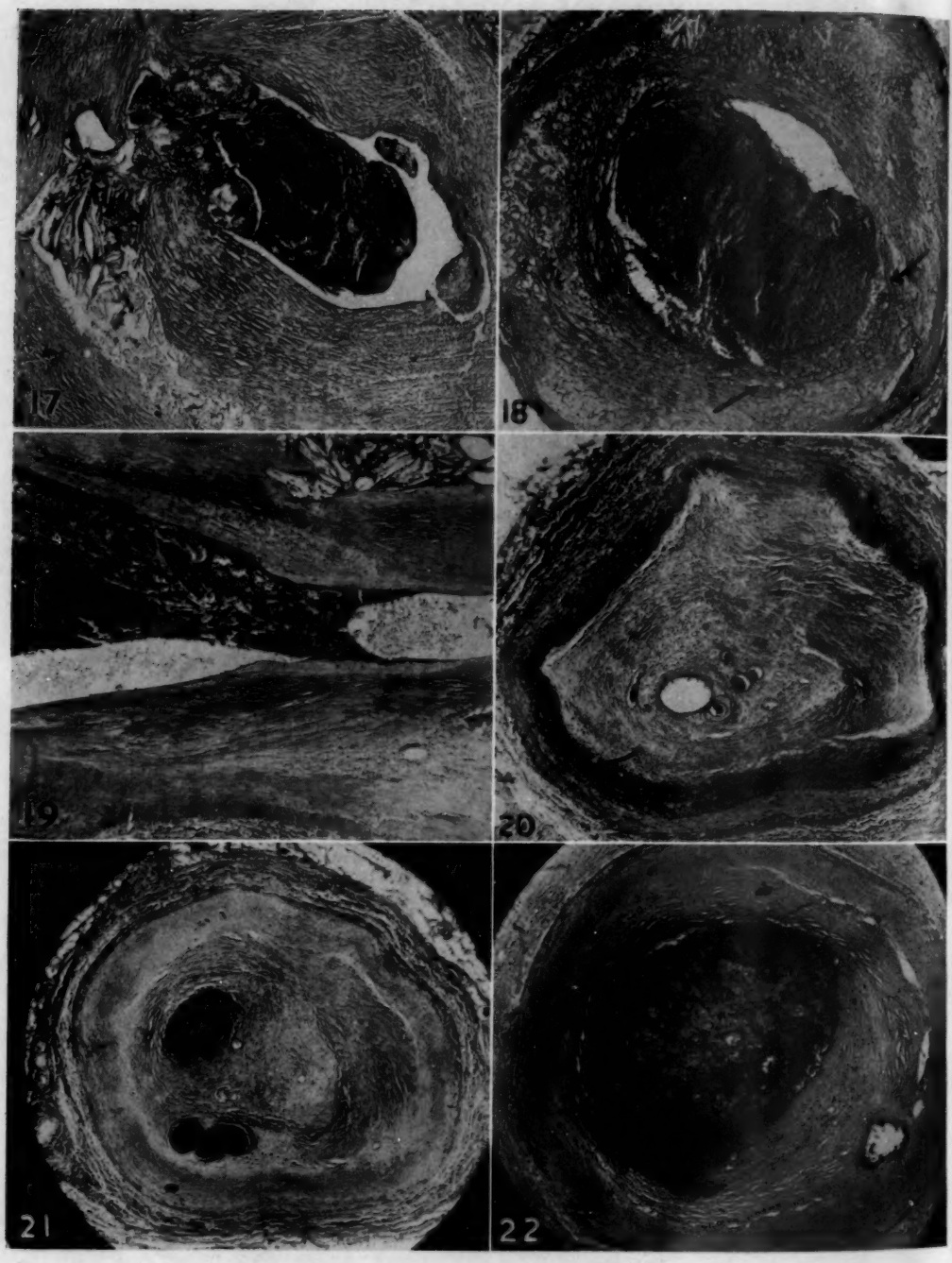
section, massive hemorrhage into the plaque became visible. Toward the distal end of the thrombus the lumen of the vessel became progressively smaller. An atheromatous zone appeared with an inner fibrous lining which was at first intact. Distally the fibrous lining of the atheroma became progressively thinner until finally a gap appeared. Through this gap in the fibrous lining of the atheroma the thrombus appeared to be continuous with a mass of fibrinoid material occupying the lipoid zone (fig. 17). At this level the thrombus had lost its cellular content, and it exhibited masses of platelets and a network of fibrin. It was attached to the wall of the vessel over the gap in the fibrous layer, and at this point the thrombus was composed of shreddy fibrin and masses of platelets. The fibrin-staining material in the lipoid area assumed the configuration of delicate anastomosing threads, outlining the acicular spaces of the atheroma; a few red blood cells and polymorphonuclear leukocytes were encountered, and no argyrophilic fibrils were seen. More distally the thrombus had disappeared, and the lumen had become still further reduced.

Group B.—In two cases (cases 8 and 9) the initially formed thrombus was deposited on an atheromatous plaque the inner fibrous lining of which was thinned out and the component collagenous fibers were widely separated. Leukocytes, red blood cells and fibrillar fibrin were present in the interstices of this rarefied fibrous lining and in the subjacent atheroma (fig. 18). Case 8 was that of a senile man who entered the hospital in a semistuporous state with left hemiplegia. Death occurred eleven days later without the appearance of additional symptoms. The ninth patient died after an illness of eighteen days characterized by pain in the right side of the chest, dyspnea and physical signs of consolidation of the upper lobes of both lungs.

Illustrative of this group is the following protocol.

CASE 8.—The postmortem examination in this case revealed hypertrophy and dilatation of the heart, severe coronary atherosclerosis, thrombosis of the left circumflex artery, sclerotic occlusion of the posterior descending branch of the right coronary artery, hemorrhagic infarction of the myocardium, thrombosis of a branch of the right pulmonary artery, lobular pneumonia and arteriosclerosis cerebri with cortical atrophy and hemorrhagic infarction of the right postcentral gyrus.

The left circumflex artery was cut serially at intervals of 15 microns over the area of thrombosis (about 3 mm.). Throughout its course the thrombosed vessel exhibited severe atherosclerosis, and in the distal sections the lumen was severely diminished. Proximally the thrombus consisted of red blood cells and fibrin reticulum, loosely packed and only partially filling the lumen. About 600 microns from the proximal end, at the point at which a large branch was given off, the thrombus compactly filled the lumen and was made up of masses of platelets, leukocytes, fibrin and red blood cells. Distally the masses of platelets rapidly disappeared, and for the rest of its course (about 1.5 mm.) red blood cells and fibrin reticulum, compactly filling the lumen, governed the pattern. Throughout its course the vessel exhibited large lipoid plaques, separated from the lumen and the thrombus by a central layer of fibrous tissue. However, frequently the fibrous layer was thinned out; the collagenous fibers were widely separated and infiltrated by many red blood cells and leukocytes (fig. 18). Pyknotic polymorphonuclear leukocytes became exceedingly numerous in the distal sections and were present not only in the subendothelial layer but deeply in the atheromatous portion. In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EXPLANATION OF FIGURES 17-22

Fig. 17.—Coronary thrombus in case 2. Note the gap in the fibrous lining of the atheroma through which fibrin-staining material in the atheroma is continuous with the thrombus. Phosphotungstic acid and hematoxylin stain.

Fig. 18.—A thrombus in the circumflex branch of the left coronary artery (case 8). A fresh thrombus fills the lumen of the vessel. The fibrous lining of the atheroma is thinned out in the area between the arrows, and blood elements have infiltrated between the separated collagenous fibers. Hematoxylin and eosin stain.

Fig. 19.—A longitudinal section through the distal end of the coronary thrombus in case 6. There is severe stenosis of the lumen of the vessel at the distal end of the thrombus. The fibrous lining of the plaque appears intact. Fibrin-staining material is visible in the underlying atheroma. (The atheromatous zone, distally, approaches the endothelium.) Phosphotungstic acid and hematoxylin stain.

Fig. 20.—Section of the distal end of the coronary thrombus in case 10 which exhibits almost complete organization and recanalization. Weigert elastic and Van Gieson stains.

Fig. 21.—The same vessel as that shown in figure 20, 300 microns proximal to the preceding section. The organization is less advanced. Phosphotungstic acid and hematoxylin stain.

Fig. 22.—The same vessel as that shown in figure 21, 750 microns proximal to the preceding section. The thrombus exhibits organization at its periphery, and the outline of the original lumen is apparent. Hematoxylin and eosin stain.

some sections the collagenous fibers of the inner fibrous layer appeared broken and interrupted. This was particularly marked in the most distal sections, where dense collections of pyknotic leukocytes were noted within the thrombus, infiltrating between such collagenous fibers and extending into the lipoid plaques. No organisms were demonstrable with the Gram-Weigert technic. In many sections fibrin-staining substance was seen to be limited to the lipoid zones, presenting a homogeneous, lumpy or fibrillar configuration outlining the acicular spaces and accompanied with red blood cells and leukocytes.

Group C.—In this group were three cases (cases 5 to 7) in which neither a fresh break nor other regressive changes were visible in the inner fibrous lining of the atheroma beneath the thrombus. The thrombi were deposited on atherosclerotic plaques the inner fibrous lining of which was apparently intact (fig. 19). In all three cases there was congestive heart failure at the time the thrombosis occurred. In case 5 death occurred nineteen hours after a sudden increase in dyspnea and cyanosis, with a progressive fall in the blood pressure. In case 6 death occurred suddenly after the patient was hospitalized for mild congestive heart failure, and in case 7 death occurred in a patient with congestive heart failure after a gradual decline over several weeks. None of the thrombi in this group exhibited organization.

Illustrative of this group is the following protocol.

CASE 6.—Postmortem examination revealed moderate hypertrophy and dilatation of the heart, severe atherosclerosis of the coronary arteries with atherosclerotic occlusion of the right and recent thrombosis of the circumflex branch of the left coronary artery, old and recent infarction of the myocardium and arteriolar nephrosclerosis.

The thrombus was about 6 mm. in length. Serial longitudinal sections were made of the thrombosed vessel at intervals of 125 microns. The vessel exhibited signs of moderately severe atherosclerosis. Large lipoid plaques were separated from the lumen by dense fibrous tissue with calcified areas. The lumen was almost completely filled by a thrombus composed of masses of platelets, a network of fibrin, red blood cells and leukocytes. There was no organization. Some longitudinal sections revealed that the distal end of the thrombus was situated at the point where the lumen had suffered the greatest narrowing (fig. 19). The subendothelial tissue, with which this portion of the thrombus was in contact, was densely fibrous. More deeply situated was a large atheroma in which fibrin-staining material presenting a coarsely fibrillar configuration outlining the acicular spaces was seen, unaccompanied with either red blood cells or leukocytes. Though here the lipoid plaque was separated from the thrombus by a broad layer of dense collagenous tissue, distally, beyond the point of origin of the thrombus, the lipoid plaque became superficial and approached the subendothelial zone.

Group D.—In this group were included two cases (cases 4 and 10) in which organization of the thrombus was too far advanced to permit recognition of the intimal lesion which existed at the time the thrombus was formed. Case 10, however, was of value because it illustrated the slow propagation which a coronary thrombus may sometimes undergo. The patient entered the hospital in a comatose state, and

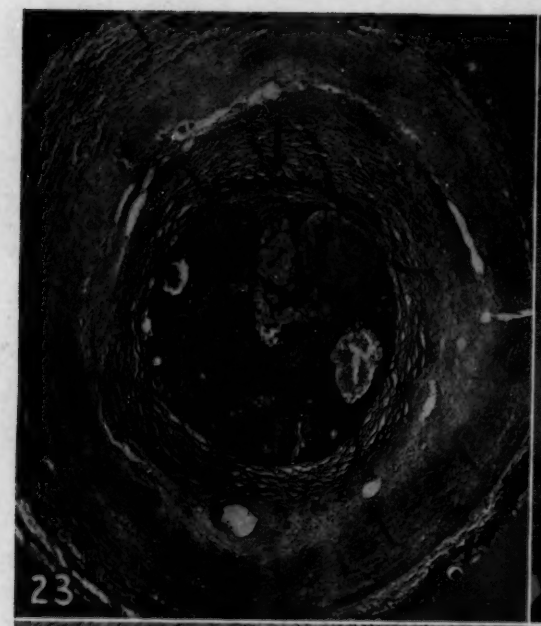
death occurred two days later, with clinical and bacteriologic evidence of meningococcic meningitis. The thrombus was about 8 mm. in length. At its distal end it was almost completely organized (fig. 20), but proximally it exhibited a gradually diminishing degree of organization (figs. 21 and 22). At the proximal end the original lumen of the vessel was readily recognized, and organization was of slight degree (fig. 23). The protocol of this case follows.

CASE 10.—Postmortem examination in this case revealed acute purulent leptomeningitis, severe coronary atherosclerosis, organizing thrombus of the anterior descending branch of the left coronary artery and infarction of the myocardium.

The thrombosed area was cut serially at intervals of 15 microns. At the distal end the occluded vessel exhibited the following features: The intima was limited externally by a wide elastic hyperplastic layer, internal to which were lipoid deposits. These deposits were covered by a broad concentric layer of dense hyaline fibrous tissue, which was sharply demarcated from a central zone of loose young connective tissue, possessing hemosiderin granules, hyaline fibrin-staining masses, red blood cells and several channels lined by endothelium and elastic tissue (fig. 20). As the sections were followed proximally the outlines of the original lumen became more clearly defined. The connective tissue elements gradually diminished, and instead was seen a progressively increasing mass of densely packed hemolyzed red blood cells and hyaline fibrin-staining material, which exhibited invasion by fibroblasts and argyrophilic reticulum (figs. 21 and 22). Finally, the central zone presented the form of a spherical mass sharply circumscribed by the dense fibrous inner layer of the intima. Fibrin-staining material was encountered within the fibrous layer, at a point about 4 mm. from the distal end of the thrombus and extending proximally for 400 microns (fig. 23). The fibrin-staining masses presented an appearance which was identical with the hyaline fibrin-staining masses of the thrombus, with which continuity was at times clearly apparent. Red blood cells were present within such areas. In the silver-stained sections the fibrin-staining masses were outlined or traversed by fine and coarse black fibrils which were continuous with fuchsinophilic fibers.

In one case (case 11) a fresh thrombus was superimposed on an older thrombotic deposit consisting of multiple hyalinized and organizing fibrin layers. The patient experienced a sudden attack of substernal pain sixteen hours before admission to the hospital and died in acute respiratory distress within a few minutes after admission. Serial sections of the vessel proximal to the occluded zone revealed severe atherosclerosis with surface and deeper fibrin-staining bands, surrounded by fibrous tissue containing young fibroblasts and argyrophilic fibrils (fig. 24). Slightly distal to this zone the partially stenosed lumen of the vessel was occluded by a fresh thrombus platelet which was approximately 700 microns in length (fig. 25). The detailed protocol follows.

CASE 11.—The patient was a man aged 48. Postmortem examination revealed severe atherosclerosis of the anterior descending branch of the left coronary artery, with occlusion of the lumen and slight hypertrophy and marked dilatation of the left ventricle. Microscopic examination of the myocardium revealed a focal subendocardial miliary infarct and an overlying mural thrombus.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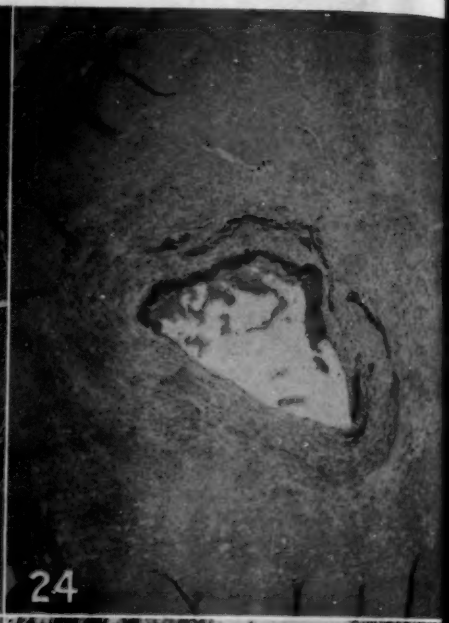
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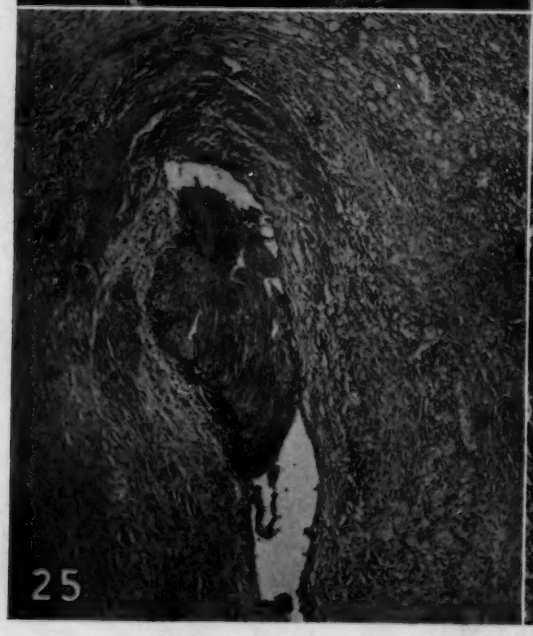
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EXPLANATION OF FIGURES 23-26

Fig. 23.—The same vessel as that shown in figure 22, 950 microns proximal to the preceding section. The arrow points to a crescentic fibrin-staining mass in the fibrous tissue in the region of the organizing thrombus. Phosphotungstic acid and hematoxylin stain.

Fig. 24.—Coronary thrombus in case 11. Section through the anterior descending branch of the left coronary artery. Note the surface and subsurface fibrin-staining masses (older parietal thrombi) which appear black in the photograph. There is a fresh deposit of platelets. Phosphotungstic acid and hematoxylin stain.

Fig. 25.—The same vessel as that shown in figure 24, 350 microns distal to the preceding section. The lumen is severely stenosed and contains a fresh thrombus of platelets. Note the cellular character of the tissue surrounding the gray subsurface fibrin-staining masses. Hematoxylin and eosin stain.

Fig. 26.—The posterior descending branch of the right coronary artery in case 8. An eccentric fibrin-staining mass is visible on the surface of the atherosclerotic plaque. Note the slender collagenous bridge partially covering the mass. Phosphotungstic acid and hematoxylin stain.

The anterior descending branch of the left coronary artery, about 1 cm. from its origin, appeared occluded by firm tissue which on transverse section presented a gray central and a yellow peripheral area. The area of occlusion did not exceed 3 mm. in diameter. This occluded area, as well as several millimeters of the vessel proximal and distal to it, was sectioned serially at intervals of 5 microns. Microscopic study disclosed the presence of a thrombus within the portion of the vessel which grossly appeared occluded. The thrombus extended over an area approximately 700 microns in length. For about 2 mm. distal to the thrombus the vessel exhibited a diminutive lumen. Proximal to the thrombus the lumen of the vessel was only moderately stenosed. In the sections containing the thrombus the vessel exhibited marked intimal thickening; the lumen was diminished to a narrow centrally placed aperture, measuring about 0.2 by 1.3 mm. The thickening of the intima appeared to be due to atheromatous plaques, largely covered by a layer of connective tissue. A layer of homogeneous fibrin-staining material was observed occupying an irregular circumferential position in the central portion of the vessel (figs. 24 and 25). It was seen extending to the lumen and lined by endothelial cells; elsewhere it was directly beneath the thrombus and separated it from the collagenous lipoid regions. Finally it was seen extending obliquely through the thickened intima so that it occupied a position in which a layer of collagenous tissue separated it from the thrombus or from the lumen of the vessel. It varied in size from a layer 250 microns in thickness to that of a few fragmented structures not exceeding the width of a single collagenous fiber. In its deeper portion, particularly where it was separated from the lumen or from the thrombus by collagenous fibers, few cells were encountered, and these were generally fibroblasts or small clumps of poorly staining distorted red blood cells. Where the fibrin-staining mass was in direct contact with the thrombus, the cellular content was increased, and pyknotic nuclei and red blood cells became increasingly numerous. The connective tissue immediately contiguous with the fibrin-staining masses exhibited numerous plump young fibroblasts, a few lymphocytes and red blood cells. Young fibroblasts were encountered also at the base of and invading the thrombus. A few capillaries also were seen. Silver stains disclosed the presence of coarse argyrophilic fibers at the border of these "fibrinoid" masses. Occasionally the "fibrinoid" masses were traversed by black fibers also, generally of a more delicate nature. For the greater part of its extent the thrombus consisted chiefly of platelets and fibrillar fibrin, with few red blood cells and leukocytes.

Comment.—The value of the study of thrombosed coronary arteries by serial sections is attested by the foregoing observations. Since the lumen of a severely affected atherosclerotic coronary artery may finally become occluded by a thrombus only 700 microns in length (case 11), the question is raised whether the presence of thrombosis can be excluded in such vessels by the examination of a few random sections.

The observations made in connection with case 10 demonstrate the value of serial sections in determining the age of a thrombus. The degree of organization may vary widely, depending on the region of the thrombus from which the sections are derived. This case also illustrates the slowly progressive propagated character of some coronary thrombi. Levy and Bruenn [15] have pointed out that in cases of coronary

15. Levy, R. L., and Bruenn, H. G.: J. A. M. A. **106**:1080, 1936.

thrombosis accompanied by sudden death the microscopic appearance usually indicates that the thrombus had formed at least several days before the heart ceased to beat. May not the slowly progressive propagation of the thrombus, with occlusion of the mouths of an increasing number of collateral branches, be held accountable for the interval between the time of the initial thrombosis and death?

What is the nature of the intimal changes underlying coronary thrombi? Leary [4] has emphasized the importance of the occurrence in some instances of coronary thrombosis of "regions of fibrinous or fibrinoid necrosis" arising in the fibrous tissue of intimal plaques and extending to the endothelial layer. In the first report of these studies it was pointed out that similar fibrin-staining or "fibrinoid" masses occur in the fibrous regions of the plaques of atherosclerotic aortas and of syphilitic aortitis and that frequently they form the base on which fresh parietal thrombi are formed. Evidence was presented which led us to the belief that these "fibrinoid" areas in most instances represent compressed and hyalinized remnants of organizing and frequently repeated surface deposits of fibrin, a view previously expressed by Mallory.[2] It was also suggested that in other instances if there is ulceration of the plaque fibrin-staining masses may represent coagulated blood plasma which has penetrated into the plaque from the lumen of the vessel. No evidence could be found to support the view that these deposits represent altered or necrotic fibrous tissue.

In the study of the coronary sections in case 11 further evidence was obtained that the fibrin-staining or "fibrinoid" masses represent the remnants of repeatedly deposited and partially organized parietal thrombi comprised chiefly of fibrin, and that on such a base of an older partially organized parietal thrombus a fresh occluding thrombus of more orthodox character may form. The occurrence of parietal thrombi on the plaques of atherosclerotic coronary vessels has been noted by others (Karsner,[16] Wolkoff,[17] Koch and Kong [18]). In case 8 the posterior descending branch of the right coronary artery presented an eccentric surface deposit of fibrin showing beginning organization (fig. 26).

It was pointed out also that as a result of repeated deposition of fibrin on the surface of plaques and its organization the plaques of syphilitic aortitis and atherosclerosis may undergo a progressive increase in size. The observations in case 11 suggest that when this occurs in the coronary arteries a slowly progressive occlusion of the lumen of the vessel ensues.

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16. Karsner, H. T., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, p. 431.

17. Wolkoff, K.: Beitr. z. path. Anat. u. z. allg. Path. **82**:555-596, 1929.

18. Koch, W., and Kong, L. C.: Beitr. z. path. Anat. u. z. allg. Path. **90**: 21-84, 1932.

In other instances when fibrin-staining material appeared in the plaque of thrombosed coronary arteries it was confined within the lipoid zones of the intimal plaques or in regions of organization. In atherosclerotic coronary arteries, as in the aorta, fibrin-staining material of a fibrillar or homogeneous form is frequently observed in the lipoid regions of plaques in the presence or absence of thrombosis, and it appears to be due to penetration of blood elements.

Benson [13] has described breaks in the integrity of the intima of thrombosed coronary arteries, which sometimes permit the escape of blood between or into the coats of the artery, and he has considered that such changes may lead to thrombus formation. Koch and Kong,[18] in their study of serial sections of thrombosed coronary arteries, also gained the impression that blood masses were pressed into the atheroma at the site of thrombosis, where ulceration of the plaque or regressive changes in its inner fibrous lining were frequent. Leary [4] has emphasized the observation of rupture of atheromatous pockets and the penetration of blood, with thrombosis occurring in the coronary arteries of older persons. On the other hand, Boyd [19] interpreted the fibrinous material in the atheromatous plaques of two thrombosed coronary arteries as an exudate appearing as a part of an acute inflammatory change within the plaque and accompanied with leukocytes.

The cases presented in this report illustrate that an occluding thrombus may form in a coronary artery under diverse circumstances. A common observation was the presence of a fresh break in the fibrous lining of a lipoid plaque with penetration of blood elements into the atheroma. In other instances thrombus formation occurred on an atheromatous plaque in which thinning out and separation of the collagenous fibers of the lining of the plaque also had permitted infiltration of blood elements. Another type observed was that in which fibrin (and perhaps other blood elements) had been repeatedly deposited on the intimal plaque before organization of the initial or subsequent deposits was completed. The lumen of the vessel was severely diminished, partly as the result of atherosclerotic changes and partly because of the progressive increase in the thickness of the plaque accompanying organization of the surface deposits; in the lumen an occluding thrombus had formed.

It is noteworthy that in all instances in which organization was not too far advanced to preclude reconstruction of the process severe diminution of the size of the lumen of the vessel was demonstrable at the site of initial thrombosis or immediately distal to it. It appears likely that the alteration of the blood flow in such vessels may be a significant factor in permitting the formation of a thrombus when a

19. Boyd, A. H.: Am. J. Path. 4:159-166, 1928.

suitable change, i. e., ulceration or erosion, in the plaque has occurred. In the aorta, where a swiftly moving blood stream exists in a widely patent vessel, the formation of an occluding thrombus is rarely observed, although the occurrence of ulcerated or eroded plaques is common.

When the blood flow in the partially stenosed vessel suffers further retardation by the appearance of congestive failure, thrombosis may occur even though the lining of the underlying intimal plaque remains intact. This is consistent with the observations of Koch and Kong.[18] They expressed the belief that in the absence of ulceration or regressive changes in the fibrous inner lining of the plaque "cardiac failure can lead to thrombosis of a more static type in a vessel whose lumen is already stenosed."

SUMMARY

Homogeneous masses which exhibited the tinctorial properties of fibrin were frequently encountered on the surface and within the superficial fibrous regions of intimal aortic plaques of atherosclerosis and syphilitic aortitis.

In the lipoid zones of aortic plaques material which stained like fibrin frequently occurred in a fibrillar or homogeneous form. This material was commoner in ulcerated or eroded plaques, but it was present also in sections of plaques which appeared intact.

Homogeneous masses staining like fibrin were observed forming a layer between the formed elements of the thrombi and the plaques in eight of nine cases of parietal aortic thrombosis. In many instances identical masses were encountered within the fibrous or lipoid zones of the underlying plaques.

The evidence is reviewed which leads us to the belief that the homogeneous fibrin-staining ("fibrinoid") masses occurring on the surface of fibrous plaques or the fibrous covering on atheromas, represent compressed and hyalinized blood elements and that the subsurface "fibrinoid" masses in most instances are the remnants of an organizing surface deposit. The "fibrinoid layer" beneath aortic thrombi represents laminated surface deposits of blood elements which have undergone a variable degree of organization.

It is suggested also that in other instances in which there is ulceration of the plaque or a loss of endothelial lining and a loosening and separation of the superficial collagenous fibers, the subsurface fibrin-staining masses may represent coagulated blood plasma which has penetrated the plaque from the lumen of the vessel.

As a result of repeated deposition of blood elements on the surface of the plaques and progressive organization of such hyalinized elements, the plaques of syphilitic aortitis and atherosclerosis may undergo a progressive increase in size. A thrombus of formed elements and orthodox

configuration may frequently supervene on such laminated, hyalinized and partially organized surface deposits.

No differences could be discerned between the tinctorial behavior of these "fibrinoid" masses and that of the fibrinous component of thrombi. It is believed that these masses owe their tinctorial properties to their fibrinous component. There is no evidence at present to support the view that the deposits of homogeneous fibrin-staining material in the intimal aortic plaques of atherosclerosis or syphilitic aortitis represent altered or necrotic collagenous fibers.

Study of serial sections of eleven thrombosed coronary arteries has revealed differences in the character of the intimal plaque at the site of initial thrombosis. In some a fresh break in the inner collagenous lining of the atheroma was demonstrated; in others the fibrous lining was thinned out, and the collagen fibers were widely separated. In the presence of congestive heart failure the thrombi were deposited on intimal plaques which were apparently intact. In one case a fresh thrombus was deposited on a plaque containing partially organized surface deposits of blood elements.

We could find no evidence to support the view that the fibrin-staining material in the plaques of coronary arteries represents altered or necrotic fibrous tissue. As in the plaques of atherosclerotic and syphilitic aortas, such fibrin-staining masses either represent the remnants of an organizing surface deposit of fibrin or are due to the penetration into the plaque of blood elements.

CHRONIC GASTRIC ULCER FOLLOWING BILATERAL VAGOTOMY IN THE RABBIT AND IN THE DOG

JAMES M. BEAZELL, B.S.
AND
A. C. IVY, M.D.
CHICAGO

A number of investigators [1] have observed the occurrence of chronic gastric ulcer in the stomach of the rabbit after bilateral subdiaphragmatic vagotomy. Donati [2] failed to obtain ulcer and expressed the belief that the ulcers observed were spontaneous. This suggestion is not tenable on the basis of our observations, since we have examined the stomachs of more than one hundred rabbits without noting an instance of chronic ulcer. Donati's failure to observe ulcer is best explained by the assumption that he did not section all the branches of the vagus nerves.

The incidence of ulcer following vagotomy reported by the various observers varied from 20 to 50 per cent, which may be explained, as observed later, by the time of survival after operation; i. e., the longer the rabbits survive the operation, up to an optimum, the greater is the incidence of ulcer.

We were interested in ascertaining the rôle that roughage plays in the genesis of ulcer of this type, since with the usual laboratory diet the rabbit's stomach is constantly filled with roughage and vagotomy induces gastric stasis. To answer this question we sectioned the vagus nerves subdiaphragmatically in three groups of rabbits. To one group the ordinary laboratory diet was fed; to a second, okra, which is rich in plant mucilage, and to a third, a diet containing no indigestible residue. The incidence of ulcer in the three groups was observed. Observations on the incidence of gastric ulcer in dogs subjected to bilateral vagotomy just above the diaphragm and fed a soft diet were also made.

From the Department of Physiology and Pharmacology, the Northwestern University Medical School.

1. (*a*) van Yzeren, W.: De pathogenese van de chronische maagzweer, Leyden, C. Kooyker, 1901; translated, Ztschr. f. klin. Med. **43**:181, 1901. (*b*) Ophüls, Wilhelm: J. Exper. Med. **8**:181, 1906. (*c*) Zironi, G.: Arch. f. klin. Chir. **91**: 662, 1910. (*d*) Greggio, Ettore: Arch. de méd. expér. et d'anat. path. **27**:533, 1916. (*e*) Alvarez, W. S.; Hosoi, K.; Overgard, A., and Ascanio, H.: Am. J. Physiol. **90**:631, 1929.

2. Donati, M.: Arch. f. klin. Chir. **73**:908, 1904; Sperimentale, Arch. di biol. **58**:323, 1904; translated, Zentralbl. f. Chir. **31**:346, 1904.

METHOD OF INVESTIGATION

Operation was performed aseptically, with the animal under ether anesthesia. A midline incision was made, and the esophagus was lifted into view. All visible nerve fibers were sectioned, and a circular area on the wall of the esophagus was then denuded of peritoneum to insure the destruction of all small vagus branches. The incision was then closed. No difficulty was encountered in the operation. Only one of the fifty-seven rabbits on which this operation was performed failed to survive the operation. The animals appeared to be normal within twenty-four hours. One group (table 1) of twenty-nine rabbits was given the standard ration, consisting of carrots, bread, oats and alfalfa. The second group of eight rabbits was fed okra (Abelmoschus esculentus) exclusively because of its high content of plant mucilage. A third group of nineteen rabbits was fed a diet consisting of 78 per cent white flour, 21 per cent dried whole milk and 1 per cent dried yeast, made into a loaf with the use of a mixture of one part of aqueous extract of carrots and one part of tomato juice. When the stomachs of the animals in the third group were examined at autopsy, only a fluid content free from indigestible residue was observed.

RESULTS OF INVESTIGATION

Rabbits Subjected to Vagotomy and Fed a Stock Diet.—Table 1 shows the results obtained with twenty-nine rabbits while on the stock diet. Analysis of these results demonstrates the following significant facts: The incidence of ulcer for

TABLE 1.—*Results of a Stock Diet on the Production of Gastric Ulcer in Rabbits on Which Vagotomy Had Been Performed*

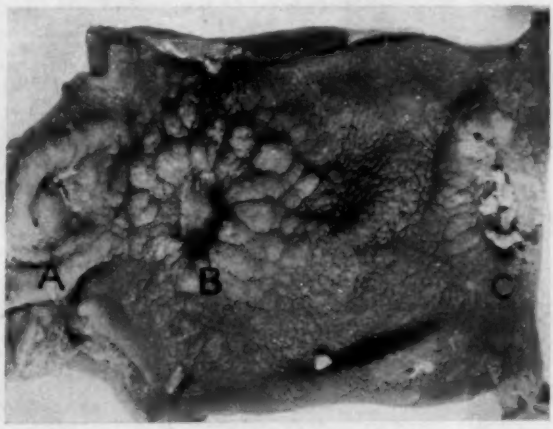
Rabbit	Length of Life After Operation, Days	Ulcer	Rabbit	Length of Life After Operation, Days	Ulcer
1	7	Absent	16	52	Present
2	7	Present	17	52	Present
3	8	Absent	18	52	Absent
4	13	Absent	19	52	Present
5	13	Present	20	55	Absent
6	14	Absent	21	60	Absent
7	15	Absent	22	81	Present
8	16	Absent	23	81	Present
9	17	Absent	24	92	Absent
10	17	Present	25	92	Present
11	19	Absent	26	87	Present
12	31	Absent	27	94	Present
13	32	Absent	28	103	Absent
14	44	Absent	29	117	Present
15	51	Absent			

the entire series was 41.6 per cent. When the animals with the shorter postoperative length of life are progressively disregarded, the following figures are obtained: If all the periods of survival of less than twenty days are disregarded, the incidence of ulcer is 50 per cent; if all of less than forty days, 56.2 per cent, and if all of less than fifty days, 66.6 per cent. The maximum incidence is apparently reached at this age. Thus, it seems that if ulcer is destined to develop in an animal, it will appear within fifty days.

The ulcers observed after seventeen days were of the typical chronic type, varying from 3 to 20 mm. in diameter. In four rabbits the lesion was single, and in the remaining eight, multiple. The greatest number of ulcers noted in a

single animal was four. In all cases the ulcers were observed on the lesser curvature of the stomach, between the incisura and the pyloric sphincter. In some of the animals with the longest period of survival after operation the ulcers showed signs of healing, as indicated by papillomatous proliferation of the mucosa (figure) about the ulcer, but in no case was the ulcer healed. In rabbits with ulceration of the stomach of longer duration than twenty days, the mucosa of the lesser curvature surrounding the ulcers definitely manifested the appearance of catarrhus verrucosus (Aschoff [3]) or *état mamelonné* (Stoerk [4]) or status mamilaris (Tandler [5]). In all the animals which died within thirty days the stomach was abnormally distended, being as much as twice the normal size in a few instances. This was true whether or not ulceration was present. It was not true after fifty days.

Dogs Subjected to Vagotomy and Fed a Soft Diet.—In the course of other investigations in this laboratory the vagus nerves of sixty dogs were sectioned above the diaphragm. The dogs were kept for from three months to a year after operation. Neither gastric nor duodenal ulcer was observed in any of these



Photograph of a portion of the stomach and duodenum of rabbit 16 showing ulcers. The area *A* in the duodenum is an apparent defect due to a fold in the duodenum; *B*, a typical ulcer, and *C*, another ulcer at the incisura, filled in part with débris. Both the ulcers are located along the lesser curvature.

dogs. It is of interest to note in this connection that of twenty dogs in which either bilateral splanchnicectomy or celiacectomy was performed, chronic duodenal ulcer developed in two.

Rabbits Subjected to Vagotomy and Fed a Diet of Okra.— It appeared probable that the difference between the rabbit and the dog with respect to the incidence of ulcer following vagotomy might be explained on the basis of differences in the diets. The dogs in our laboratory are maintained on a soft diet of relatively low residue, while the standard rabbit diet is high in roughage. Since, as is well

3. Aschoff, Ludwig: Pathologische Anatomie: Ein Lehrbuch für Studierende und Aerzte, ed. 6, Jena, Gustav Fischer, 1923, vol. 2.

4. Stoerk, O.: Wien. klin. Wchnschr. **35**:855, 1922.

5. Tandler, Julius: Lehrbuch der systematischen Anatomie, Leipzig, F. C. W. Vogel, 1923, vol. 2, p. 131.

known, the rabbit stomach is never empty, it appeared likely that the continuous irritation of a rough gastric mass, in the presence of abnormal motor conditions, might be a factor in the production of ulcer. Fauley and Ivy [6] demonstrated the marked influence that the consistency of the diet has on the chronicity of acute ulcer in the rabbit. Meek [7] observed ulcer in two of thirteen vagotomized dogs showing gastric stasis while on a diet of dog biscuit, even after several months. This indicates that the character of the diet is a factor in the production of ulcer after vagotomy.

This hypothesis was tested by placing a series of vagotomized rabbits on a soft diet. Eight animals were fed a diet consisting exclusively of okra. The animals in this series died on the eighteenth, nineteenth, twenty-third, twenty-sixth, twenty-sixth, twenty-ninth, thirty-ninth and fortieth day, respectively. At autopsy no ulcer was observed. If in the group used as a control (rabbits on the stock diet) all the animals which died or were killed before the eighteenth or after the fortieth day are disregarded, nine animals are left, with an incidence of ulcer of 22.2 per cent. Thus, in at least one of the rabbits which were fed okra ulcer should have developed. This series is obviously too small to justify any unqualified conclusions.

TABLE 2.—*Results of a Nonresidue Diet on the Production of Gastric Ulcer in Rabbits on Which Vagotomy Had Been Performed*

Rabbit	Length of Life After Operation, Days	Ulcer	Rabbit	Length of Life After Operation, Days	Ulcer
3	9	Absent	7	31	Absent
4	9	Absent	11	31	Absent
15	12	Absent	17	45	Absent
5	14	Absent	8	53	Absent
6	17	Absent	2	54	Absent
12	17	Absent	18	60	Absent
1	21	Absent	19	61	Present
14	24	Absent	9	68	Present
16	29	Present	10	68	Absent
13	30	Absent			

Rabbits Subjected to Vagotomy and Fed a Diet With No Residue.—Since the rabbits could not be maintained on the okra, feeding of the soft synthetic diet was resorted to.

Vagotomy was done on nineteen rabbits which were then placed on this diet. The results are shown in table 2. When animals on the soft diet are considered as one series, these results are obtained: When all animals which died before the twenty-ninth postoperative day are disregarded, eleven animals are left, with an incidence of ulcer of 27.2 per cent. When all the animals of the group used as a control (fed a rough diet) which died before the twenty-ninth day are disregarded, the incidence of ulcer is 50 per cent for eighteen animals. If the rabbits fed okra and those fed the soft diet are considered as one group, only three of fourteen animals which survived twenty-nine days or longer had ulcer—an incidence of 21.4 per cent. Because of the definite difference, it appears that a comparison of the series receiving the soft diet and that receiving the rough diet is justified. It is thus apparent that changing the character of the diet from rough to soft decreases the incidence of ulcer after vagotomy by about 50 per cent.

6. Fauley, G. B., and Ivy, A. C.: Arch. Int. Med. **46**:524, 1930.

7. Meek, W. J.: Personal communication to the authors.

COMMENT

The location of the ulcers which occurred in the rabbits after vagotomy is significant. In every instance they were observed along the lesser curvature in the pyloric antrum, the place in which, with few exceptions, gastric ulcer is seen in man.

The occurrence of the ulcers in this area is most adequately explained in two ways. 1. It is in this prepyloric region of the stomach that peristalsis is most active. As stated by Aschoff, this is the area where lines of force converge. Consequently, it is here that there is the greatest likelihood of traumatization under abnormal conditions of motility. 2. Berlet [8] has shown that the blood supply to the region of the *Magenstrasse* is more likely to be influenced by vascular changes than that elsewhere in the stomach. The vessels of this region are sparser and more tortuous and contain fewer anastomotic intercommunications than the vessels elsewhere in the stomach.

Owing to technical difficulties, the effect of vagotomy on gastric secretion in the rabbit has not been investigated. However, it is well known that in the dog bilateral vagotomy is followed by a period of paralytic hypersecretion [9] lasting from ten to fourteen days, after which the amount of secretion drops markedly to a subnormal level and then slowly returns to the normal level over a period of months.[10] If one accepts the probability that the same response holds true for the rabbit, hyperacidity cannot be considered a factor in the production of ulcer, since the maximum incidence does not occur until after the period of paralytic hypersecretion has ended. Indeed, the maximum incidence of ulcer comes during a period that in the dog is notable for its diminished secretion. Ophüls [1b] tested the postmortem acidity of the gastric contents of the animals in his series and found it to be normal both in the rabbits with ulcer and in those without.

Although the control, if any, exerted by the vagus nerves on the blood vessels of the stomach has never been demonstrated, it has been suggested that these nerves antagonize the vasoconstrictor action of the splanchnic nerves. If true, the constrictor effect of the splanchnic nerves after vagotomy would be unopposed, resulting possibly in impairment of the blood supply to the stomach. Yet such an explanation is not likely in view of the fact that Alvarez and his colleagues [1e] observed gastric ulcer in rabbits not only after vagotomy but after splanchnicectomy and

8. Berlet: Ztschr. f. Path. **30**:472, 1924.

9. Orbeli, L. A.: Arch. di sc. biol. **12**:71, 1906. Babkin: Die äussere Sekretion der Verdauungsdrüsen, Monographien aus dem Gesamtegebiet der Physiologie, Berlin, Julius Springer, 1928. Tomaszewski: Arch. f. d. ges. Physiol. **171**:1, 1918.

10. Hartzell, J. B.: Am. J. Physiol. **91**:161, 1929. Vanzant, F. R.: ibid. **99**:375, 1931.

after the combination of the two operations. All the operations caused changes in motor activity.

The most obvious explanation of the occurrence of ulcer after section of any nerve supplying the stomach is that ulcer results from trophic disturbances. However, the fact that ulcer does not occur in all portions of the stomach seems to eliminate trophic change as a significant possibility. It would be difficult to imagine trophic disturbances confining themselves entirely to isolated areas of the stomach. Even though ulcer was attributed to such a disturbance, little would be gained.

The possibility that ulcer is due to denervation of the muscularis mucosa was investigated by Ophüls.[1b] It seemed possible that the normal response of the mucosa to injurious objects in the gastric contents—i. e., pulling away—might be impaired by section of the vagus nerves. He found that this reaction was not affected, whether the tests were performed immediately or weeks after the nerve had been sectioned.

The fact that ulcer still occurred when the animal was on a soft diet, although the frequency was reduced one-half, shows that mechanical irritation is a factor, but not the sole factor, concerned in the genesis of ulcer. That gastric stasis was present in the animals on a rough diet was evident at autopsy. Meek and Herrin [11] observed gastric stasis after several months in vagotomized dogs when they were fed solid but not liquid food. The importance of a rough diet in delaying the healing of acute ulcer of the stomach in the rabbits was demonstrated by Fauley and Ivy.[6]

SUMMARY

Bilateral subdiaphragmatic vagotomy was performed on thirty rabbits. Of the twenty-nine animals that survived the operation and that received a rough diet, twelve had gastric ulcer. The maximum incidence, 66 per cent, occurred in the animals which survived fifty days or more after the operation. The incidence for the rabbits which survived longer than twenty-nine days was 50 per cent. The ulcers were located on the lesser curvature, between the incisura and the pyloric sphincter, and were of typically chronic type.

Of the nineteen vagotomized rabbits which received a soft, nonresidue diet, ulcer developed in only three. The incidence of ulcer in the rabbits which survived longer than twenty-nine days was 27 per cent. The soft diet decreased the incidence of ulcer by about one-half.

Thus, mechanical trauma in association with gastric stasis was a factor, but not the sole factor, in the genesis of ulcer of this type. The other factor is unknown.

11. Meek, W. J., and Herrin, R. C.: Am. J. Physiol. **109**:221, 1934.

Ulcer did not occur in sixty dogs which were subjected to bilateral vagotomy above the diaphragm and fed a soft diet. Meek [7] reported the occurrence of two instances of gastric ulcer in thirteen vagotomized dogs which were fed dry dog biscuit, under which conditions gastric stasis results.

Case Reports

ISLET ADENOMA IN THE PANCREAS OF A MOUSE

W. C. Hueper, M.D., Wilmington, Del.

While an appreciable number of benign and malignant islet tumors of the pancreas have been observed during recent years in man, this type of neoplasm is apparently rare in animals. So far only 2 instances have been placed on record in the literature. Both were in dogs (Bru [1] and Slye and Wells [2]). In view of the rarity of this neoplastic process among animals in general and of the marked infrequency of pancreatic tumor in mice in particular (Slye, Holmes and Wells [3] found 2 pancreatic tumors among 20,000 spontaneous neoplasms in 125,000 mice), the following case of islet adenoma of the pancreas in a white mouse is reported.

The mouse belonged to a group of mice which were killed after having been exposed for approximately four months to the inhalation of vapors of carbon disulfide in low concentration. The autoptic and histologic observations of the various organs examined (the lungs, heart, liver, spleen, stomach, intestine, adrenal glands and kidneys) were essentially normal, with the exception of the condition of the pancreas, to be described.

The section of the pancreas showed to the naked eye a well circumscribed, round, light-stained area measuring about 1.5 mm. in diameter embedded in the dark pancreatic tissue stained blue-purple.

On microscopic examination the small nodule appeared to be surrounded by a delicate fibrous capsule. It was composed of a solid mass of cells rather regular in shape and size with a light pink-stained cytoplasm and dark round nuclei, arranged in indistinctly outlined strands and clusters and showing the same tinctorial and morphologic qualities as the cells of the pancreatic islets. A moderate number of mitotic figures was present. Two small cystic areas filled with homogeneous pink-stained matter and surrounded by islet cells were noted in the central portions of the tumor. A small amount of a fine fibrillar substance was seen between the cell strands. The stroma was scanty and consisted almost entirely of the cellular lining of the blood capillaries. Some of the islets in the

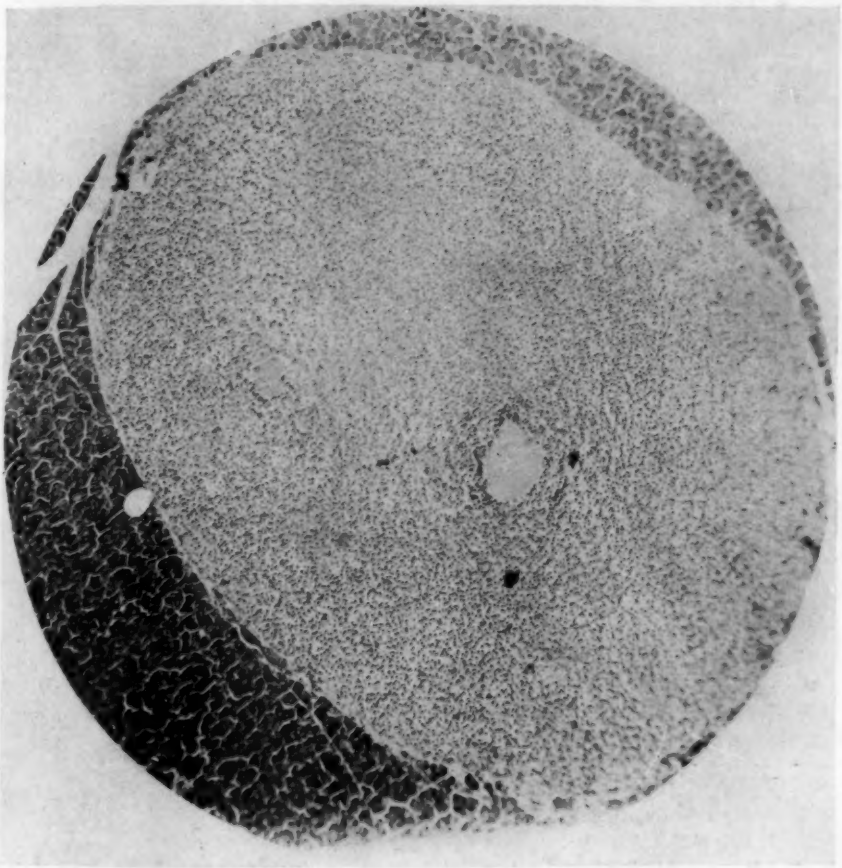
From the Haskell Laboratory of Industrial Toxicology.

1. Bru, P.: Rev. méd.-chir. d. mal. du foie **2**:40, 1927.
2. Slye, M., and Wells, H. G.: Arch. Path. **19**:537, 1935.
3. Slye, M.; Holmes, H. F., and Wells, H. G.: Am. J. Cancer **23**:81, 1935.

surrounding pancreatic tissue were abnormally large, reaching from two to three times the normal size. The pancreatic tissue of external secretion was normal in appearance. There were a few islands of fat tissue scattered throughout the gland.

COMMENT

As the instance reported represents an accidental observation, there are no data available as to the possible metabolic effects of this tumor.



Islet adenoma in the pancreas of a white mouse; low magnification.

In view of the coexistence of hypertrophic islets in the pancreas of the mouse, this case resembles to a certain extent that recorded by Slye and Wells, in which many adenomas were present in the pancreas of a dog, 2 of which showed malignant qualities.

PAPILLARY FIBROMA OF CARDIAC VALVE

AMBROSE J. HERTZOG, M.D.,* MINNEAPOLIS

Small papillary tumors of the cardiac valves are of interest because of their rarity and the confusion that exists concerning their structure and classification. They represent incidental observations at necropsy and apparently produce no symptoms.

Lambl [1] in 1856 described fine threadlike excrescences on the aortic cusps. He found these excrescences in 2 per cent of one thousand cases. Curtis [2] in 1871 seems to have been the first to describe a tumor of a cardiac valve. This tumor was a small papillary fibroma. He considered it to be inflammatory in origin, as an associated endocarditis was present. Since then a number of authors have reported what they considered true neoplasms on the cardiac valves. Branch [3] in 1931 reported a fibroma of the tricuspid valve and from the literature collected reports of four other cases, including the one reported by Curtis. Fibroma of these valves has also been reported by Simmonds,[4] Quickert,[5] Kornfeld [6] and Celotti.[7]

The majority of authors who have reported tumors on the valves of the heart considered the tumors to be myxoma. Jaleski [8] in 1934 reported a case of myxoma of the tricuspid valve. In an extensive review of the literature he collected reports of twenty-two other instances. In this group, however, he included several of fibroma. Jalesky believed these instances of myxoma represented a group with uniform morphologic characteristics. He was uncertain whether they were degenerative processes in connective tissue growths or true neoplasms. Galassi [9] in 1915 described a tumor of the aortic valve that he considered to be myxofibroma. Forel [10] in 1919 reported a case of endothelioma of the mitral valve and considered it to be the first of its kind observed.

REPORT OF A CASE

The tumor was discovered in the course of an autopsy on an 81 year old man who died of carcinoma of the rectosigmoid with generalized carcinomatosis.

The heart weighed 340 Gm. The epicardium appeared normal. The ventricular walls were of normal thickness. The appendages and endocardium were normal.

From the Department of Pathology, University of Minnesota.

* Mayo Foundation Fellow in Pathology.

1. Lambl, V. A.: Wien. med. Wchnschr. **6**:244, 1856.

2. Curtis, M. B.: Arch. de physiol. norm. et path. **4**:262, 1871.

3. Branch, C. F.: Am. J. Path. **7**:157, 1931.

4. Simmonds, M.: München. med. Wchnschr. **55**:1154, 1908.

5. Quickert, quoted by Husten, Karl: Beitr. z. path. Anat. u. z. allg. Path. **71**:132, 1923.

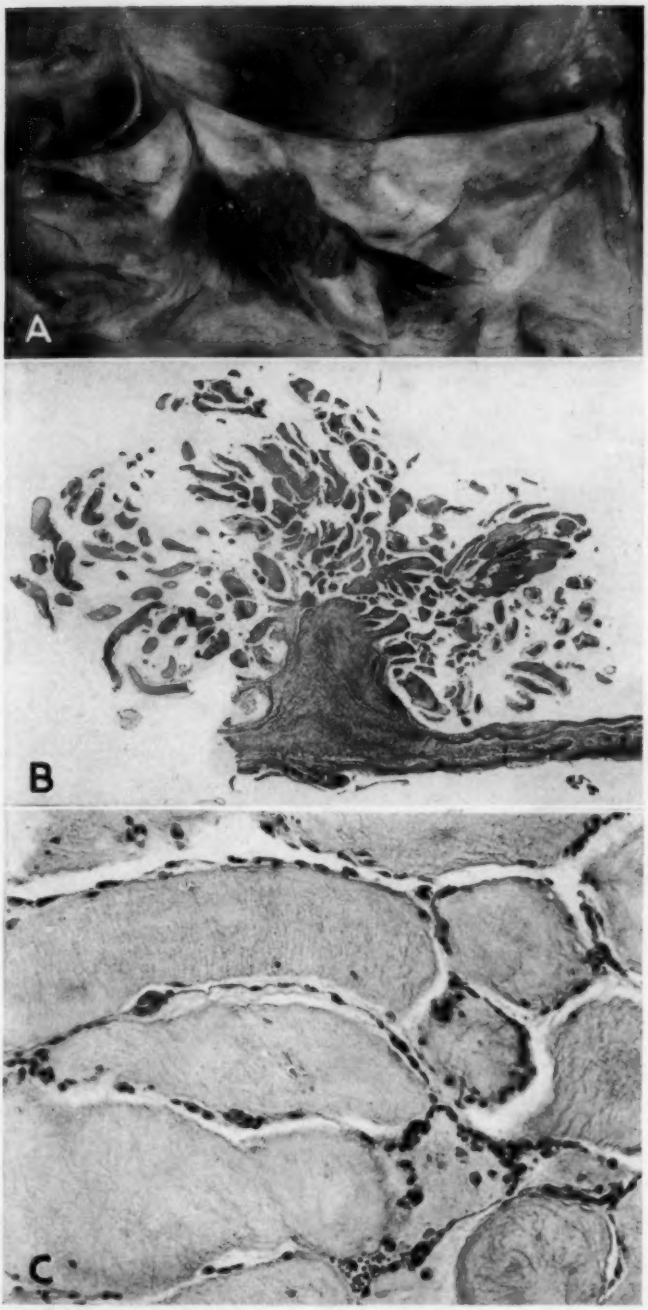
6. Kornfeld, Marcel: Virchows Arch. f. path. Anat. **270**:873, 1929.

7. Celotti, Mario: Pathologica **21**:613, 1929.

8. Jaleski, T. C.: Am. J. Path. **10**:399, 1934.

9. Galassi, Carlo: Pathologica **7**:501, 1915.

10. Forel, F. C.: Internat. Clin. **4**:147, 1919.



A, photograph of a papillomatous growth on the aortic valve; *B* and *C*, photomicrographs of papillary processes arising from short pedicle (*B*) and covered with endothelium (*C*).

On the middle cusp of the aortic valve was a small pedunculated growth measuring 1.5 by 1 by 1 cm. It was composed of fine delicate processes arising from a short pedicle, which was firmly attached to the surface of the valve (*A*, figure). There was no evidence of associated endocarditis. The remaining valves were normal.

Microscopically, the tumor consisted of numerous branching processes and had a short thick pedicle. The papillary tufts were covered with a layer of endothelium, which in some areas appeared to have several layers. When stained with hematoxylin and eosin the pedicle and processes had a homogeneous pink matrix (*B* and *C*, figure). Stained with azocarmine they were seen to consist of a fine fibrillar fibrous stroma. There was no evidence of inflammation of the valve.

SUMMARY

This case is similar to the previously reported ones of fibroma. It is also similar histologically to some of the reported cases of myxoma. However, mucous tissue could not be demonstrated. A diagnosis of myxoma is not justified. Whether this tumor is an excrescence as described by Lambl or a true neoplasm is debatable. Koechlin [11] concluded that primary neoplasms of the cardiac valves have never been proved to exist and that the reported ones were forms either of a Lambl excrescence or of an inflammatory reaction. In this case there is no evidence of endocarditis that would suggest an inflammatory origin, and there is no similarity between the tumor and the small blood cysts which occur on the valves of the new-born. This tumor is essentially a fibrous papillomatous growth with an endothelial covering arising from the surface of the valve.

11. Koechlin, E.: Frankfurt. Ztschr. f. Path. **2**:295, 1909.

Laboratory Methods and Technical Notes

THE USE OF DIOXANE IN THE PREPARATION OF HISTOLOGIC SECTIONS BY THE PARAFFIN METHOD

CARL J. BUCHER, M.D.
Assistant Director of Laboratories, the Jefferson Medical College Hospital
AND
KETURAH D. BLAKELY, L.T.
Technical Assistant
PHILADELPHIA

Dioxane (di-ethylene di-oxide) was introduced by Groupner and Weissberger [1] in 1931 as a clearing and dehydrating agent in the preparation of histologic sections by the paraffin method. While not generally used in this country, it has many advantages over the older methods. The use of dioxane eliminates much of the delay and expense incident to dehydrating tissue with alcohol and clearing it with such an agent as cedar oil, chloroform or xylene. The results in general are superior to those obtained by the traditional technic. It has practically no hardening effect and is miscible in all proportions with water and melted paraffin. Fixed tissue may be transferred directly into dioxane and then into melted paraffin to infiltrate.

We have prepared five-thousand blocks of tissue by the paraffin method using dioxane as the dehydrating and clearing agent. At first the technics of Groupner and Weissberger and Mossman [2] were rigidly followed. Soon, however, certain modifications gleaned from experience with the method were made. Our method and observations follow.

TECHNIC

Fixation.—Any of the common fixatives may be used with equally good results. It is not necessary to place tissues fixed in Zenker's or Helly's fluid in alcohol after washing; they may be brought into dioxane directly. Certain objects containing colloid or colloid-like substances which have been fixed in a dilute solution of formaldehyde U. S. P. (1:10) are washed in running water for from twelve to twenty-four hours before being placed in the dehydrating agent. Unless this is done the colloid tends to stain basically when placed in hematoxylin. It is the only kind of tissue that has needed any special treatment after fixation.

Sundry Notes.—The use of calcium chloride to absorb water from dioxane is economical. When it is employed it is not necessary to renew the dioxane in the dehydrating dishes oftener than once in two weeks. Even then, the dioxane may be filtered, fresh calcium chloride may be placed in the Stender dish and the filtered reagent may be used again. With a little experience one can judge when filtration

1. Groupner, Heinz, and Weissberger, Arnold: Zool. Anz. **96**:204, 1931.
2. Mossman, H. W.: Anat. Rec. (supp.) **58**:82, 1934.

therefore seem preferable. The technic of staining, however, is influenced by the previous treatment of the tissues with the reagent.

It is important to remove all the paraffin from the tissues before staining them. Areas from which the paraffin is not completely removed will stain poorly or not at all. The sections can be stained by any method compatible with the primary fixative used, and they will stain better than they do when dioxane is not used. Among the stains which we have used are Mallory's eosin and methylene blue, toluidine blue and erythrosin, phosphotungstic acid-hematoxylin, Van Gieson's stain, Mallory's aniline blue and orange G, de Galantha's stain for Spirochaeta pallida and stains for iron and tubercle bacilli. For the guidance of those who may choose to use the method, a summary of the staining of sections by hematoxylin and eosin follows:

1. Place in xylene for from three to five minutes; repeat.
2. Rinse in a tumbler of 95 per cent alcohol, moving the slide back and forth.
3. Place in 95 per cent alcohol for from three to five minutes.
4. Rinse in tap water.
5. Place in distilled water for five minutes.
6. Stain with hematoxylin (Harris). The staining is timed according to the age and dilution of the stain and is controlled by microscopic examination of the section. Delafield's hematoxylin has not proved to be as satisfactory as that of Harris.
7. Wash in tap water.
8. Place in a 2 per cent aqueous solution of sodium hyposulfite until developed (for from fifteen to thirty seconds). This develops the color uniformly. Other reagents were tried but were found to be more or less unsatisfactory.
9. Wash thoroughly in tap water.
10. Place in tap water for five minutes or longer to develop the stain.
11. Stain with 1 per cent eosin (yellowish water-soluble) for thirty seconds. The sections stain intensely with eosin after dioxane treatment.
12. Place in 95 per cent alcohol for three minutes.
13. Place in absolute alcohol for from three to five minutes.
14. Immerse in xylene until clear (about five minutes); repeat.
15. Mount in Canada balsam in xylene.

NOTE: Sections fixed in Zenker's fluid or some other mercury bichloride fixative are placed in compound solution of iodine after step 4. The excess iodine is removed with a 5 per cent solution of sodium hyposulfite, and the staining is continued.

SUMMARY

We believe that dioxane as a clearing and dehydrating agent is superior to graded alcohols and the older clearing agents. The tissues do not shrink or become brittle, and they cut better. The staining reactions are improved. The stains of our oldest preparations have not faded. Both time and money are saved, as is shown by the fact that only 3 gallons (11 liters) of dioxane was required to dehydrate and clear five-thousand blocks of tissue and that with fixation in solution of formaldehyde the process from the time of the receipt of the unfixed tissue to the completion of the preparation is accomplished in forty-eight hours.

is necessary from the appearance of the calcium chloride, which crumbles and becomes finely divided. We employ unrefined commercial dioxane. Dr. Mossman [2] has suggested removing such water and alcohol as may be contained in the dioxane by preliminary treatment with calcium oxide and calcium chloride. Our experience has shown that to be a needless step. No attempt should be made to redistill dioxane, as it is inflammable and in the process certain explosive compounds are formed. Dioxane also evaporates readily, and the fumes produce a somewhat anesthetic effect. For these reasons all containers should be covered with a lid or kept tightly corked. The same precautions that are observed in handling acetone, xylene and other volatile and inflammable compounds are imperative.

Dehydration and Clearing.—The sections should not exceed 4 mm. in thickness. After fixation they are placed in a Stender jar, 62 mm. in diameter and 93 mm. in height, containing one part by volume of distilled water and three parts by volume of dioxane. The jar is filled three-quarters full. This preliminary step prevents shrinkage and preserves delicate structures faithfully. If speed is required, it may be omitted. One hour is sufficient time for tissues to remain in this fluid. They are then placed in a perforated porcelain cup, which we have previously described,[3] and the cup, resting on a glass support, is placed in a Stender dish containing pure dioxane. The bottom of the Stender dish is covered to a depth of 1 cm. with anhydrous calcium chloride. By elevating the tissues on the glass support they are placed at the center of the volume of fluid, and the water which comes out of them, being heavier than dioxane, sinks to the bottom of the Stender dish and is taken up by the calcium chloride. Likewise, the tissues are prevented from coming into direct contact with the calcium chloride. The material is left in the fluid from two to three hours. Further prolongation of the time in this fluid does no harm.

Infiltration.—The tissues are transferred directly from the dioxane to a paraffin bath. Here again, it is necessary to keep the tissues well elevated, for the dioxane will sink to the bottom of the container as infiltration takes place. The best results are obtained by using copper wire gauze as a platform, thereby allowing better circulation of fluid than is afforded by the use of the porcelain cups, and at the same time the copper does not harm the material. Groupner and Weissberger [4] commented on the fact that with many objects the tissue fell out of the ribbon. We have overcome this difficulty completely by prolonging the time of infiltration from eight to twelve hours or overnight. The paraffin employed has a melting point of from 56 to 58 C. Several changes of paraffin are unnecessary. As the dioxane is volatile, the bath cleanses itself.

Mounting, Blocking and Cutting.—These steps are performed as usual. The cutting is much improved by the method, since tissues do not become brittle as they do after dehydration with alcohol. Materials such as thyroid gland, spleen, adipose tissue and tissue containing large amounts of blood, which ordinarily are difficult to section, cut easily, and the resulting preparations are thinner and more nearly perfect. For routine study sections are cut 5 microns thick, although thinner ones are readily obtained.

Staining.—We have departed radically from technics employing dioxane in staining the tissues. Dioxane is slightly acid when tested with litmus paper, and if it is employed here the structures in the preparations lack definition and sharpness. Time is not saved, and the fumes are somewhat unpleasant. The older methods

3. Bucher, Carl, and Blakely, Keturah: Arch. Path., to be published.

4. Groupner, Heinz, and Weissberger, Arnold: Zool. Anz. **102**:39, 1933.

General Review

INFLAMMATION IN RESISTANCE TO INFECTION

ARNOLD RICE RICH, M.D.

BALTIMORE

It is a pleasure to take part in a symposium that unites immunologists, pathologists and bacteriologists, for I strongly believe that one of the most needed developments in the study of bacterial infection today is a more active and pointed endeavor to coordinate the material of these three sciences. While pathology, bacteriology and immunology meet most obviously in the study of inflammation, which is the subject of the present symposium, the interrelationship of these sciences is by no means confined to the local inflammatory process. They are no less interrelated in all the manifold reactions of the body to bacterial infection. Fever, constitutional symptoms, tissue damage and destruction, leukocytosis and leukopenia, acute splenic tumor and alterations in general metabolism and in the specific functions of various organs—all these and numerous other disturbances which make up the variegated picture of bacterial disease—are problems urgently demanding a more closely correlated analysis of their pathologic, bacteriologic and immunologic interrelationships.

A well developed resistance to infection, whether native or acquired, manifests itself in three fundamentally important ways. First, the bacteria are prevented from spreading freely through the tissues from the site at which they lodge; second, the progressive multiplication of the bacteria is inhibited, and most or all of the organisms that penetrate into the tissues are destroyed, and, finally, any injurious substances that the bacteria are capable of liberating either before or after their death are rendered less noxious or even completely harmless. In what ways and to what degree does the inflammatory mechanism assist in the accomplishment of these ends? In the present paper, I cannot, of course, hope to cover or even mention all the important aspects of this complex mechanism. I have chosen, therefore, to limit my discussion chiefly to a consideration of the manner in which some of the

From the Department of Pathology and Bacteriology, the Johns Hopkins University School of Medicine.

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constituents of the inflammatory exudate contribute to the localization and the destruction of infecting bacteria. I realize that there are still unsolved problems relating to almost every aspect of this question, and I wish to say that this review is presented with no intent of dogmatism. It constitutes only an attempt to outline what appears to me to represent the present limits of knowledge concerning some of the defensive potentialities of the inflammatory reaction.

It has long been obvious that the increased flow of fluid from the blood vessels into the tissues during inflammation can serve the beneficial purpose of diluting, and so rendering less irritating, any injurious substances liberated by the infecting bacteria. In addition to this simple diluent effect, in the immune body the fluid that exudes from the vessels may contain specific, neutralizing antitoxin, and antibody may also cause the local precipitation and consequent immobilization of soluble, injurious bacterial substances, comparable to the local fixation of foreign animal protein in the tissues of a body containing specific precipitin for that protein, as demonstrated some years ago by Opie.[1] The antibody of the exudate may also bring about the immobilization of intact bacteria in a manner to be discussed later, and, in addition, it assists in the destruction of the bacteria either through its power to render virulent micro-organisms phagocytosable (opsonization) or by its direct bactericidal effect in the case of the bacteria for which specific lysins can be developed. Furthermore, since for a time at least there is a markedly increased flow of lymph from the affected area, it is clear that the site is flushed and that irritating products of cellular destruction are washed away by a moving stream of fluid.

While local benefits are conferred by the exaggerated movement of fluid, it is no less clear that untoward effects also may result from it. In the first place, the bacteria themselves are often swept through the tissues on the moving wave of inflammatory edematous fluid, and the zone of infection is thereby widened, to the detriment of the infected body. When the movement of fluid is assisted by the force of gravity this adverse effect may be greatly exaggerated, as has been demonstrated by Rhodes and Goodner [2] and as my associates and I have had frequent opportunity to confirm under a variety of circumstances. Furthermore, since particulate matter that enters the tissues or body cavities passes freely into the lymphatics, as was demonstrated some years ago by MacCallum,[3] the moving stream of inflammatory fluid likewise carries infecting bacteria directly into the lymphatic channels, and the

1. Opie, E. L.: J. Immunol. **17**:329, 1929.
2. Rhodes, C. P., and Goodner, K.: J. Exper.Med. **54**:41, 1931.
3. MacCallum, W. G.: Bull. Johns Hopkins Hosp. **14**:105, 1903.

spread of infection to the blood stream by this route is, of course, common.

In addition to this untoward effect of the inflammatory edema, the accumulation of an excessive amount of fluid in the tissues can act to prevent the contact of bacteria with the phagocytes of the exudate and so may permit their unimpeded growth. Leukocytes cannot swim through fluid with any degree of effectiveness. They must have a framework on which to crawl. And this brings one to a consideration of a second important mechanical effect of the fluid of the inflammatory exudate, namely, its property of clotting to produce fibrin.

Certainly, one of the important effects of the formation of a fibrin network in the tissues is the fact that it provides interlacing pathways that bridge in all directions across the fluid-distended tissue spaces and on which the phagocytes can move more readily toward the infecting bacteria.

A second important function which the coagulation of the exudate performs is in assisting in the prevention of spread of bacteria from the infected site. The effect of fibrin in walling off the organisms is obvious and familiar in localized infection of the pleural and peritoneal cavities, but even within the substance of a tissue the clotting of the fluid portion of the exudate assists in preventing the spread of bacteria which are enmeshed in the coagulum. When inflammation is well established after infection with many bacteria, the histologic study of the infected area often shows plainly the presence of an encircling barrier of fibrin and leukocytes, which manifestly prevents the free escape of the bacteria from the site. It is not only when an obvious fibrinopurulent circumscribing wall is present, however, that the spread of the bacteria may be inhibited. It has long been known that if certain bacteria are injected into an area in which diffuse inflammation has first been induced by an irritant even to only a moderate degree, the bacteria spread from the site less readily than from a previously normal site. Menkin,[4] in an instructive series of experiments dealing with the mechanism of this inhibition of spread of bacteria from an area of prepared inflammation, reached the conclusion that it is due primarily to the mechanical effect of the network of fibrin in the tissues and to the occlusion of the efferent lymphatics by fibrinous thrombi. He offered evidence that bacteria differ in their ability to provoke early deposition of fibrin, and he suggested that the localization or spread of a given bacterium will be determined to a significant degree by the ability of that bacterium to incite the formation of a fibrinous exudate or to cause thrombosis of the local lymphatics.

4. Menkin, V.: Am. J. M. Sc. **190**:583, 1935.

I think that there can be no doubt that widespread thrombosis of lymphatics and a dense coagulum in the tissue spaces must, when they are present, interfere markedly with the drift of bacteria from the infected site and that this action of fibrin must be regarded as one of the important protective potentialities of the inflammatory exudate. There are, however, reasons for believing that mechanical obstruction is by no means the whole story of the localizing power of an inflamed area, and this, I feel sure, Menkin himself never intended to question. In the first place, Menkin [4] stated that broth injected directly into the tissues does not produce any appreciable deposit of fibrin or lymphatic thrombosis, and yet others have shown that even so mild an inflammation as that produced by applying a sterile broth compress to the surface of the skin causes the localization of certain bacteria deposited in the inflamed area, with a striking limitation of the resulting lesion as compared with the extent of the lesion produced by the infection of a normal area.[5] Furthermore, as was shown by Cohnheim fifty years ago [6] and as was recently studied again more precisely by Field, Drinker and White,[7] there exists from the beginning a great increase in the flow of lymph from an inflamed area, and at the height of the inflammation the lymph flow is still greater than that from a normal area. Why, then, do not the bacteria continue to be swept freely from the site by this increased movement of fluid, even though some lymphatics may be thrombosed? That the increased lymph flow drains the area of inflammation is clear, for Bezançon and Labbé [8] and Menkin and Freund [9] have shown that at the height of inflammation produced by staphylococci or sterile irritants the lymph draining from the area contains many polymorphonuclear leukocytes—cells which are not present in normal lymph. One may properly ask, therefore, why it is that bacteria are not swept freely through the interstices of the fibrinous network in the tissues and lymphatics by the increased movement of fluid if the far larger leukocytes are. Must one assume that wherever bacteria are present in an inflamed area the immediately adjacent lymphatics are occluded and that the leukocytes drain only from other parts of the inflamed area where the lymphatics are open? Certainly, our own examination of the relation of bacteria to the lymphatics in multiple sections through several hundred experimental inflammatory lesions would not permit such an

5. Freedlander, S. O., and Toomey, J. A.: J. Exper.Med. **47**:663, 1928.

6. Cohnheim, J.: Vorlesungen über allgemeine Pathologie, Berlin, A. Hirschwald, 1877, vol. 1, p. 211.

7. Field, M. E.; Drinker, C. K., and White, J. C.: J. Exper. Med. **56**:363, 1932.

8. Bezançon, F., and Labbé, M.: Arch. de méd. expér. et d'anat. path. **10**: 318, 1898.

9. Menkin, V., and Freund, J.: Arch. Path. **8**:263, 1929.

assumption. Lymphatic thrombosis occurs, of course, but except in instances of the most violent inflammation I have never been able to satisfy myself that it is widespread enough to account for the retention of bacteria at the site. May it not be, rather, that there are also factors associated with the physicochemical alterations in an inflamed area which tend to promote the adsorption or adherence of certain bacteria and certain foreign substances to the fibrin and the tissues? Menkin, for example, reported that ferric chloride fails to drain freely from an inflamed area into which it is injected, and this he interpreted as being due primarily to a fibrinous network and to lymphatic thrombosis.[10] It is not improbable, however, that the fixation of ferric chloride in an inflamed area may be due more to the fact that, since iron tends to be precipitated with protein, the high protein content of the inflammatory exudate favors the precipitation of the injected iron and so inhibits its free drainage from the site. Menkin also reported that when horse serum is injected into an inflamed area in a rabbit the diffusion of the serum from the area is inhibited, and this, too, he regarded as due to a fibrinous network and to lymphatic thrombosis. He,[10] however, mentioned the fact that marked coagulation occurs when horse serum is added in vitro to the fluid obtained by centrifugating the previously well clotted inflammatory exudate. Certainly, if such a marked coagulum forms in vivo when horse serum is injected into an inflamed area, it provides an important reason that the serum does not diffuse freely from the site, regardless of the state of patency of the lymphatics. Menkin has shown that trypan blue is likewise held in inflamed areas, and he expressed the belief that this also is due to mechanical blockage by fibrin and lymphatic thrombosis.[10] It appears important, however, to examine this phenomenon in the light of the work of Grollman,[11] who showed that colloidal dyes are strongly adsorbed by body proteins and that the degree of adsorption varies with the p_H of the medium. It is interesting that Grollman found that the blood proteins of the rabbit (the animal used by Menkin) adsorbed colloidal dye to a much more marked degree than those of the dog or other animals studied. In view of these facts and the known marked difference between inflammatory exudates and normal lymph (and undoubtedly also normal tissue fluid) in protein content, hydrogen ion and electrolyte concentration and various other important ways, it is highly desirable that the specific physical and chemical conditions present in a given area of inflammation should be carefully examined in relation to the nature and electrical charge of any test material injected into it for their possible influence on any observed tendency of the area to hold at the site the test material,

10. Menkin, V.: J. Exper. Med. **53**:171, 1931.

11. Grollman, A.: J. Biol. Chem. **64**:141, 1925; Am. J. Physiol. **75**:287, 1926.

whether bacteria, protein, electrolyte, colloidal dye or particulate matter. That such factors can exert an important effect on the localization or spread of foreign substances is not only reasonable theoretically but evident from a consideration of the Duran-Reynals phenomenon, i. e., the remarkable ability of testicular extract to promote the spread of bacteria and nonliving substances when injected with them into the tissues. The recent evidence that certain bacteria produce a material that exerts a similar effect on tissue permeability [12] is interesting in this connection. Undoubtedly, as I shall point out later, in the immune body the local fixation of bacteria is due primarily to specific physical factors rather than to simple mechanical obstruction by deposits of fibrin.

But aside from physical conditions and mechanical obstruction, it is apparent that additional factors are involved in the localizing and protective action of an area of prepared inflammation, for the area acts to inhibit not only the spread of the bacteria but the free proliferation of certain organisms; they are destroyed, and the area is sterilized. These properties of an area of prepared inflammation have been studied by numerous investigators during the past forty years, and this inhibition of the growth and life of the bacteria must be regarded as an important factor in the observed ability of an inflamed area to prevent progressive spread of a lesion and generalized infection when certain types of bacteria are injected into it. If the multiplication of the bacteria is inhibited by the prepared inflammation there is obviously less opportunity for the production of a large, spreading lesion and the degree of invasion of the lymphatics and blood stream will be correspondingly decreased. While mechanical blockage by fibrin, and other factors, can play a rôle in temporarily restraining the spread of bacteria, the degree of suppression of the growth of the bacteria will always play a dominant rôle in determining the size of the lesion, the degree of invasion of the blood stream and the ultimate outcome of the infection. This, undoubtedly, accounts for the well known fact that not all types and strains of bacteria are localized effectively by an area of prepared inflammation. If the particular bacteria used in the experiment cannot be destroyed by the mobilized phagocytes and are able to proliferate freely under the conditions obtaining in the inflamed area, progressive spread of the local lesion, septicemia and death will unfailingly occur, regardless of the amount of fibrin in the tissues or the lymphatics. In the case of bacteria which can be ingested by the body's phagocytes, it is obvious that their growth can be readily suppressed when they are injected into an area in which an enormous number of leukocytes have first been mobilized. But certain bacteria which cannot be readily phagocytosed

12. McClean, D.: J. Path. & Bact. **42**:477, 1936.

may also be held in check by an area of prepared inflammation if they are not injected in too large numbers, and in these instances it appears that the physicochemical conditions at the site are unfavorable to the growth and life of the bacteria. It has been shown by Kempner and Peschel [13] and others that in an inflamed area there may be marked oxygen deficiency, and the amount of carbohydrate may fall to a very low level; the accumulation of lactic acid, lowering of the bicarbonate content and other factors lead to the development of local acidosis, and additional important changes occur. It is desirable to develop the study of the nature and effects of the physicochemical conditions present in inflamed areas in relation to the growth and survival requirements of each particular pathogenic bacterium, for, as I have said, it is well known that not all types and strains of bacteria are effectively localized by an area of prepared inflammation. Loebel, Shorr and Richardson [14] have made an admirable beginning in this direction in relation to the tubercle bacillus.

Mechanical factors which tend to inhibit bacterial spread can operate effectively only in the face of bacteria which can be destroyed by the body's defenses, whether native or acquired. In the case of bacteria for which the body possesses natural opsonin and which therefore can be readily destroyed by the phagocytes, the mechanical barrier of fibrin is really of little moment in protection, for in most instances of this sort, unless massive numbers are injected, the bacteria are phagocytosed and destroyed before they can produce marked deposits of fibrin or a lesion of appreciable size and the bacteria that escape to the regional lymph nodes are promptly phagocytosed and destroyed there. In the body with a high degree of acquired immunity, a barrier of fibrin is likewise of subsidiary importance, for here too the bacteria will be quickly destroyed and, in addition, the body with acquired immunity possesses a new and potent mechanism of bacterial localization, which I shall discuss presently. A mechanical barrier is of importance chiefly in the defense against bacteria to which the body possesses only an imperfect degree of resistance but yet a resistance which can operate successfully if adequate time is provided. If such bacteria can be kept localized, they may eventually be destroyed at the site, while if they are permitted to spread they may produce lesions in distant organs, or even fatal infection.

It is of interest in this connection to point out that there are bacteria for which the body possesses no natural opsonin and which can proliferate freely for a time within the body, even to the extent of

13. Kempner, W., and Peschel, E.: Ztschr. f. klin. Med. **114**:439, 1930.

14. Loebel, R. O.; Shorr, E., and Richardson, H. B.: J. Bact. **26**:139 and 167, 1933.

producing large inflammatory lesions and bacteremia, but which will always be eventually destroyed. Miss McKee and I have recently been engaged in a study of the mechanism of this type of resistance, and I shall briefly mention these experiments because they bear on the question of bacterial localization. It has long been known that, although rabbits are extremely susceptible to the type I pneumococcus, they are highly resistant to almost all strains of encapsulated type III pneumococci. Although it has been stated that the normal rabbit's leukocytes cannot ingest the latter strains of type III pneumococci in vivo [15] or in vitro,[15a] we have found that smooth strains that are virulent for mice but avirulent for rabbits are eventually phagocytosed under proper conditions both in vivo and in a mixture of rabbit serum and leukocytes. When injected intracutaneously some of these strains produce in the rabbit only a relatively small lesion, which heals rapidly. There are, however, other strains which, if injected in the encapsulated form that neither the polymorphonuclear nor the mononuclear phagocytes of the normal rabbit will readily phagocytose, still never produce fatal infection, although the local lesion spreads progressively and attains considerable size. This peculiar phenomenon of immunity in the absence of readily demonstrated phagocytosis has excited the interest of a number of investigators, who have advanced divergent views regarding the mechanism involved in the immunity. I cannot enter here into a discussion of the details of this problem. For my present purpose I shall state only that we have made the interesting observation that within the first twenty-four hours of the infection the rabbit acquires a marked ability to restrain the growth of this pneumococcus and to destroy it. Thus, if the skin of one side of the body was inoculated with the bacteria, a large lesion that spread progressively during the first twenty-four hours always resulted. When, however, we injected the same dose into the histologically normal skin of the opposite flank at various intervals after the first infection, we were amazed to find that as early as twelve hours after the first injection the animals had already acquired a demonstrable increase in ability to resist a second infection and that at the end of twenty-four hours this resistance had reached a very high degree of effectiveness, as evidenced by the fact that the second lesion was sharply restricted in size. Cultures and inoculations of mice showed that the bacteria in the second lesion were much more rapidly destroyed than those in the lesions of the first infection.

I cannot discuss here our studies of the mechanism responsible for this rapidly acquired ability to suppress the growth of these bacteria

15. Singer, E., and Adler, H.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **41**:71 and 468, 1924.

15a. Tillett, W. S.: J. Exper. Med. **45**:1093, 1927.

further than to state that we have shown, both in vivo and in vitro, that the degree of fever of the rabbit is a highly important factor. For example, if the animal's temperature is raised artificially in a thermostat, a first infection will be effectively restricted. Nor shall I dwell on the interesting point that natural immunity and recovery in certain infections can be determined largely by the degree to which, during the course of the primary infection itself, the body acquires an ability to suppress the further growth of the infecting bacteria. I have mentioned these experiments chiefly because they illustrate the fact that a fibrin barrier is of far less importance in restraining the spread of infection than is the ability to suppress the growth of the bacteria, for since in these experiments the first infection led invariably to a widely spreading lesion, the bacterium was obviously not of a type which promptly called forth a deposition of fibrin sufficient to inhibit its own spread, and yet a few hours after infection, when the body had acquired the ability to suppress the growth of the bacteria, the lesions of the second infection were sharply localized. The results of the experiments further warn one against attributing the final cessation of spread of the first lesion merely to the development of a fibrinous network or to lymphatic thrombosis, for, while it is true that deposits of fibrin occur in the primary lesion, the sharp localization of the second infection shows that by the time the primary lesion ceases to spread the body has already acquired the power to suppress the extension of the infection by means of a different and more potent mechanism.

In the body with well developed acquired immunity, whether active or passive, the fluid portion of the inflammatory exudate may itself exert a potent and specific action in preventing the spread of bacteria. Several years ago we showed that one of the important functions of antibody is its action in holding the specific bacteria at the site at which they lodge in the tissues.[16] In the nonimmune body, virulent bacteria separate from each other after division and drift freely through the tissue spaces, but under the influence of the specific antibody they adhere to each other after division and therefore grow in clumps in the tissues, just as they are held together in the familiar skein formation when growing in serum containing antibody in vitro. The presence of agglutinin changes, in effect, a micro-organism which has a tendency to spread by separating and drifting through the tissues and lymphatics into one which grows in the tissues in relatively immobile clumps resembling staphylococci and therefore gives rise to lesions that are sharply localized, in contrast to the diffuse, spreading lesions produced by the same bacterium in the nonimmune body. The important work of Mudd, Lucké

16. Rich, A. R.: (*a*) Bull. Johns Hopkins Hosp. **52**:203, 1933; (*b*) Proc. Nat. Acad. Sc. **16**:460, 1930.

and their co-workers,[17] Northrop,[18] Shibley[19] and others has demonstrated that when bacteria become covered by antibody their surface properties are altered in a manner that causes them to adhere to each other and to the leukocytes with which they come into contact. In our studies bacteria in the immune body were observed to adhere to the fixed tissues as well and to be thus held fast at the site where they lodge. That this action of the immune body in preventing the separation and drift of the bacteria is effected by the antibody was readily demonstrated by the method of passive immunity. In both the actively and the passively immunized body this localizing action of antibody can be shown to be in operation within a few minutes after the bacteria reach the tissues, and it serves the important function of immediately immobilizing the bacteria where they lodge until the phagocytes can be brought to the site by inflammation. The process is undoubtedly similar to that shown to be in operation in Opie's studies on the local fixation of foreign protein in the body possessing precipitin. Opie was inclined to believe that the fixation of the protein was accomplished by the allergic inflammation which occurred at the site of injection under the particular conditions of his experiments.[1] In the case of bacteria, however, we have shown, by appropriately designed studies, that this localizing effect is accomplished by the antibody and that it operates effectively even in the complete absence of fibrin or inflammatory cells,[20] though in the absence of the phagocytes the bacteria proliferate progressively to enormous numbers and at length overwhelm the body, as I shall show later. The studies of Cannon and Pacheco[21] and Catron[22] confirmed the potency of this localizing effect of antibody, and these investigators agreed that antibody acts effectively in the absence of a mechanical barrier of fibrin. The importance of a mechanism which will hold bacteria fixed even at sites at which the movement of tissue fluid is increased by inflammation is obvious.

Aside from certain anatomic peculiarities of the different tissues, then, the principal factors that determine the sureness with which an infection will be localized are: first, the ability of the body to suppress the growth of the bacteria and to destroy them; second, the immobilizing action of antibody, for since antibody when present is distributed throughout the

17. Strumia, M.; Mudd, S.; Mudd, E. B. H.; Lucké, B., and McCutcheon M.: J. Exper. Med. **52**:299, 1930.

18. Northrop, J. H., in Jordan, E. O., and Falk, I. S.: Newer Knowledge of Bacteriology and Immunology, Chicago, University of Chicago Press, 1928, p. 782.

19. Shibley, G. S.: J. Exper. Med. **44**:667, 1926.

20. Rich, A. R., and McKee, C. M.: Bull. Johns Hopkins Hosp. **54**:277, 1934.

21. Cannon, P. R., and Pacheco, G. A.: Am. J. Path. **6**:749, 1930.

22. Catron, L.: J. Exper. Med. **61**:735, 1935.

tissue fluids, as shown by Freund and Whitney [23] and others, it can exert its immobilizing action on invading bacteria even before appreciable inflammation has occurred, although the supply of antibody in the tissue fluid can, of course, be increased to good advantage by that which may later escape from the blood stream with the inflammatory exudate; third, the mechanical, localizing effect of fibrin, and finally, the high probability that nonspecific physicochemical conditions in inflamed areas may influence to an important extent the degree to which bacteria of a given strain drift from or held at the site. This is a matter deserving careful study. The degree to which bacteria spread from the site at which they lodge in any instance is determined by the degree to which these various factors are brought into play in the particular body into which the bacteria gain entrance.

Although much has been written about the bactericidal power of normal blood serum, there have been fewer studies on the bactericidal power of inflammatory fluid, which is materially different from serum. Such studies as have been made by Zinsser,[24] Gay and Clark [25] and others have shown that cell-free inflammatory fluids may exert varying, and I may say inconstant, degrees of bactericidal or growth-inhibiting action on certain bacteria, and this observation Miss McKee and I had an opportunity to confirm during the course of experiments carried out several years ago. It is also known that if an extract of leukocytes is added to the inflammatory fluid the bactericidal power may be considerably augmented, though in our experience it rarely becomes highly potent.[20] These bactericidal effects observed in vitro are not always paralleled by a similar effect in vivo. Various bacteria which grow poorly or not at all when implanted in small numbers into serum or inflammatory fluid in a test tube may grow surprisingly well in the fluids of the living, infected body or, indeed, in the blood and tissue fluids of the body after death. Of course, in an immunized body in which a specific bacteriolysin is present, the inflammatory fluid may exert a highly potent bactericidal effect—as shown by the Pfeiffer phenomenon. But specific bacteriolysins or bactericidal antibodies that are effective in vivo have been demonstrated in relation to only a few bacteria, and in most instances pathogenic bacteria live and multiply even in the immunized body if phagocytosis is prevented, as will be seen later.

These facts do not mean, of course, that bacteria never, under any circumstances, die in the body unless they are ingested and destroyed by phagocytes. The extracellular death of tubercle bacilli in areas of

23. Freund, J., and Whitney, C. E.: J. Immunol. **16**:109, 1929.
24. Zinsser, H.: J. M. Research **22**:397, 1910.
25. Gay, F. P., and Clark, A. R.: Arch. Path. **1**:847, 1926.

caseation is a familiar phenomenon, and several years ago I described the extracellular death and dissolution of virulent pneumococci under certain conditions in the tissues of the immunized body.[16b] This phenomenon is sometimes observed when the bacteria, forced into colony formation by the action of agglutinating antibody, lie out of the reach of phagocytes in the center of a zone of necrotic tissue that is circumscribed by a wall of leukocytes. Growth ceases, and the individual bacteria of the colonies gradually lose their affinity for the specific bacterial stains and at length fuse together into a formless mass, which eventually undergoes solution. In such instances, however, it is clear that the death of the bacteria is not the result of a specific bactericidal property of the inflammatory fluid but is due apparently to unfavorable chemical conditions developing in the necrotic, walled-off area, comparable in part, perhaps, to the conditions which bring about the cessation of growth and the death and lysis of pneumococci that are incubated in a limited and fixed amount of nutrient medium in vitro. In the same body, in less walled-off lesions in which there is free flow of fluid, such extracellular lysis of these bacteria is rarely, if ever, seen. It may be remarked in this connection that one of the potentially beneficial results of the action of agglutinating antibody in forcing the bacteria to grow in colony formation and to adhere to the tissues is that their opportunity to escape from unfavorable local influences by drifting to sites that provide better nutrition and environmental conditions is thereby greatly reduced.

I have mentioned the desirability of studying more intensively the physicochemical conditions in inflamed areas, with a view toward possible correlations with the defense mechanism. In this connection the studies of Kempner and Peschel,[13] Rohde,[26] Menkin [27] and others have shown that as a rule inflamed areas tend gradually to acquire an acid reaction. It has long been known that in the early stages of most types of acute inflammation the polymorphonuclear cells predominate and that with the passing of time the mononuclear cells increase and eventually may outnumber the polymorphonuclears. Menkin [27] reported suggestive experiments which led him to believe that the cytologic picture of the exudate may be determined by its hydrogen ion concentration. It is true that in most types of inflammation, as time passes and the number of polymorphonuclear cells decreases, the reaction of the exudate gradually becomes acid and the number of mononuclears increases, but it is still a question whether the cellular change results from the change in hydrogen ion concentration or whether the change in p_H results from the activities and dissolution of the inflammatory and tissue cells.

26. Rohde, C.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **40**:85, 1926.
27. Menkin, V.: Am. J. Path. **10**:193, 1934.

Polymorphonuclear cells differ from mononuclear phagocytes in an important respect; the polymorphonuclear cells are unable to reproduce themselves, whereas the reproductive power of the macrophages is very great. This is probably one reason that the polymorphonuclear cells usually emigrate from the blood vessels in far greater numbers than do the mononuclears. That time is required for the appearance of large numbers of macrophages in an inflamed area may be due in part to the fact that their increase often depends largely on their own power of multiplication, and this requires time. But one is still left with the problem of what the stimulus is that incites the mononuclear cells to multiply in the inflamed area. Perhaps it is the acid reaction of the exudate, but the matter requires more direct demonstration. In the study of tissue cultures of the buffy coat of the blood, I have often been impressed by the fact that they exhibit a cellular change strikingly like that occurring in inflammatory exudates. During the first two days there is a great preponderance of polymorphonuclear cells. These cells have no power of multiplication and gradually disintegrate. Meanwhile, the monocytes have steadily multiplied, and after two or three days the number of living macrophages far surpasses that of the polymorphonuclear cells. As far as I am aware, there have been no studies correlating the cytologic characteristics of a culture of blood cells with changes in the hydrogen ion concentration, similar to the studies carried out on cultures of embryonic tissue several years ago by Lewis and Felton.[28] Studies of this nature relating to the influence of hydrogen ion concentration and other factors on the survival and death of the polymorphonuclear cells and on the multiplication of the mononuclear cells may well yield direct information bearing on the interesting and important question that Menkin has raised concerning the relationship of the changing hydrogen ion concentration to the cytologic character of the inflammatory exudate.

Before leaving the question of the reaction of inflammatory exudates, however, I wish to recall the experimental demonstration by Fenn[29] and others that the phagocytic activity of leukocytes is markedly influenced by the hydrogen ion concentration of the medium. Since definite effects were observed within a p_H range that corresponded to that occurring in inflamed areas, the possible implications in relation to antibacterial defense are obvious and merit more pointed investigation. The same may be said for variations in osmotic pressure, for marked changes in osmotic pressure occur in inflamed areas, and it has been shown in vitro that phagocytosis is definitely influenced by the tonicity

28. Lewis, M. R., and Felton, L. D.: Bull. Johns Hopkins Hosp. **33**:112, 1922.
29. Fenn, W. O.: J. Gen. Physiol. **5**:169, 1922.

of the medium. Karsner and Merrill,[30] furthermore, have shown that the locomotion of leukocytes is influenced to a considerable degree by the concentration of the salts in their surroundings.

Space does not permit me to discuss the numerous other important problems relating to the properties of the fluid portion of the inflammatory exudate, for I wish to mention briefly some problems relating to the cellular constituents.

While the polymorphonuclear neutrophil is the body's primary defense against most bacteria that penetrate into the tissues, it has been clear from the time of Metchnikoff that the macrophages also have the power to phagocytose bacteria. Indeed, the careful studies of Lucké, Mudd and their co-workers [31] revealed no significant difference between the ability of macrophages and polymorphonuclear cells to phagocytose various bacteria. In a limited number of types of infection, such as tuberculosis, the macrophages clearly play a more important rôle in defense than do the polymorphonuclear cells, although the latter cells are able to phagocytose the bacteria. Even in acute pyogenic infection the macrophages not infrequently serve as reenforcements for the polymorphonuclears, which bear the brunt of the initial attack. Furthermore, the macrophages that lie in the sinusoids of the liver and spleen play a highly important rôle in ridding the blood of bacteria which find their way into the circulation, as the studies by Cannon and his co-workers [32] and others have demonstrated. The importance of the macrophage in the defense against bacteria is therefore unquestioned. One may, however, properly question the view of certain investigators [33] that even in pyogenic infection the major credit in antibacterial defense should be given to the macrophages. While it is true that under the highly artificial conditions employed by these investigators (such as the mobilization of large numbers of macrophages at a given site before the bacteria are introduced or the injection of the bacteria into the bone marrow or the blood stream) the macrophages avidly ingest phagocytosable bacteria that are delivered to them, it is doubtful whether any one who follows histologically from moment to moment the fate of bacteria in previously uninflamed tissues can fail to appreciate the transcendent importance of the polymorphonuclear cells in the defense of the body against the great majority of pyogenic bacteria. In most types of acute

30. Karsner, H. T., and Merrill, A. T.: Arch. Path. **7**:101, 1929.

31. Lucké, B.; Strumia, M.; Mudd, S.; McCutcheon, M., and Mudd, E. B. H.: J. Immunol. **24**:455, 1933.

32. Sullivan, F. L.; Neckermann, E. F., and Cannon, P. R.: J. Immunol. **26**:49, 1934.

33. Singer and Adler.[15] Gay, F. P.: J. A. M. A. **97**:1193, 1931.

infection, if the body possesses a high degree of immunity, bacteria reaching the tissues in reasonable numbers are phagocytosed and destroyed by the polymorphonuclears before the macrophages have increased to numbers sufficient to play even an auxiliary rôle of appreciable importance. When the body possesses no resistance, neither the macrophages nor the polymorphonuclears can phagocytose the bacteria, and in spite of the most intense inflammation the proliferation and spread of the bacteria proceed uninterruptedly. It is when the body's risistance is of only moderate degree (and this is determined by a number of different factors which cannot be discussed here) that the destruction of the bacteria by the polymorphonuclear cells is delayed and time is provided for the active participation of the macrophages in the phagocytic defense, but even under this condition, if the tissues are studied continually from the very beginning of the infection, it will be observed that the macrophages serve strictly as later arriving reenforcements and not as the primary, or sole, phagocytic defense. In our own studies of hundreds of sites of experimental infection produced by pyogenic bacteria we have never encountered an instance in which phagocytosis by macrophages predominated over that by polymorphonuclear cells if the infection was induced in previously uninflamed tissues and was followed histologically from the beginning to the end—and this no matter what degree of immunity or hypersensitivity was present. In pointing this out it is not my intention to minimize the adjuvant importance of the macrophage in pyogenic infection. Indeed, there may even be exceptional instances of pyogenic infection in which the macrophages are more important than the polymorphonuclears, although I believe that it is desirable to have more positive evidence than is now possessed before the assumption is accepted as established. In mentioning the matter at this point it is my purpose chiefly to question the not infrequently encountered generalization that immunity to pyogenic bacteria depends primarily on macrophagic phagocytosis. Owing to the influence of the conclusions of investigators who have maintained this view, it is even stated today that monocytes can destroy pneumococci but that polymorphonuclears are unable to do so.[34] The phagocytosis and destruction of sensitized pneumococci by polymorphonuclear leukocytes can, of course, be readily observed in vitro.

When the leukocytes disintegrate in an area of inflammation they liberate proteolytic ferments, the nature of which has been studied by

34. (*a*) Weiss, C., and Czarnetzky, E. J.: Arch. Path. **20**:233, 1935. (*b*) Gay, F. P., and others: Agents of Disease and Host Resistance Including the Principles of Immunology, Bacteriology, Mycology, Protozoology, Parasitology and Virus Diseases, Springfield, Ill., Charles C. Thomas, Publisher, 1935, p. 453.

Müller,[35] Opie,[36] and Longcope and Donhauser[37] and others. The principal proteolytic ferment of human polymorphonuclear cells is tryptic in nature and that of the mononuclear phagocyte peptic. In addition, catheptic[34] and ereptic[38] ferments have been found in the polymorphonuclear leukocytes of animals. These ferments play an important rôle in the liquefaction of fibrin and of dead tissue preliminary to repair. It is interesting to speculate as to whether under certain conditions the ferments may exert an unfavorable influence on resistance by destroying antibody at the inflamed site, for Felton and Kaufmann[39] have shown that protective antibody is destroyed by similar proteolytic ferments. Whether the weaker ferments of the leukocytes can act rapidly enough to exert an unfavorable effect in this direction has not yet been determined.

While it can be readily shown in vitro that extracts of leukocytes possess a certain degree of bactericidal power for various micro-organisms, it is not established that it is the proteolytic ferments which are responsible for this action. Indeed, Jochmann[40] could detect no bactericidal activity in solutions containing leukocytic proteolytic ferments, and Jobling and Petersen[41] and others found living bacteria highly resistant to the action of proteolytic ferments from other sources, although dead bacteria are readily lysed by them. Precisely to what the bactericidal power of leukocytic extracts is due has not yet been determined, but whatever it is, it is not sufficiently potent to prevent progressive infection when injected into the tissues together with virulent bacteria, though when liberated in large amount into walled-off areas of inflammation it may conceivably exert its bactericidal effect to better advantage.

It is well known that bacteria differ in their susceptibility to intracellular destruction by the phagocytes. The mechanism of intracellular destruction is still obscure and warrants pointed investigation. It is interesting that the studies of Douglas[42] on cultures in vitro suggested that the ability of the leukocytes to digest various bacteria after ingestion may depend in part on sensitization of the bacteria by the serum in which they are suspended. Although Jobling and Petersen[43] have shown in vitro that sensitized bacteria are more readily digested by proteolytic

35. Müller, F., cited by Kossel: Ztschr. f. klin. Med. **13**:149, 1888.
36. Opie, E. L.: Physiol. Rev. **2**:552, 1922.
37. Longcope, W. T., and Donhauser, J. L.: J. Exper. Med. **10**:618, 1908.
38. Parker, J. T., and Franke, E.: J. M. Research **37**:345, 1917.
39. Felton, L. D., and Kauffmann, G.: J. Immunol. **13**:219, 1927.
40. Jochmann, G.: Ztschr. f. Hyg. u. Infektionskr. **71**:71, 1908.
41. Jobling, J. W., and Petersen, W.: J. Exper. Med. **20**:452, 1914.
42. Douglas, S. R.: Proc. Roy. Soc., London, s.B **89**:335, 1917.
43. Jobling, J. W., and Petersen, W.: J. Exper. Med. **20**:321, 1914.

ferment than are unsensitized bacteria, considerations in all these experiments do not permit the conclusion that the proteolytic ferment of the leukocytes is responsible for the intracellular death of the bacteria preliminary to digestion. It is conceivable that intracellular bacterial destruction may be effected less by a specific bactericidal substance than by conditions within the phagocyte that are unfavorable to the life of the bacteria. The ingested bacterium has to compete with the phagocyte in which it lies for nutrient and life-sustaining materials, and in addition the intracellular environment may be incompatible with the survival of the bacterium. Rous,[44] for example, showed that the hydrogen ion concentration of the interior of the living phagocyte may be as low as 3 or less, regardless of the reaction of the surrounding medium. This is a degree of acidity at which many bacteria cannot live. No specific bactericidal agent would be required for the destruction of an ingested bacterium if a similar degree of acidity developed in the portion of the cell immediately surrounding it.

While no one today would question the fundamental rôle played by inflammation in the defense against bacteria, the belief is still encountered from time to time that the fixed tissues of the body with acquired immunity can, in some vague and undefined manner, act independently of the phagocytes in protecting the body against infecting bacteria. At present, it is fair to state that there is no acceptable evidence for the existence of a so-called acquired tissue immunity that can protect the body independently of the action of the phagocytes and antibody. Miss McKee and I[20] showed several years ago that if animals are highly immunized, so that they are able promptly to destroy virulent type I pneumococci deposited in their tissues, and are then deprived of leukocytes, bacteria injected into the tissues will grow uninterruptedly, regardless of the degree of immunization or the amount of protective antibody present in the plasma and tissue fluids. Although the immobilizing action of antibody that I have already described holds the proliferating bacteria localized for a long time in the immunized leukopenic body, as the result of their continued growth the bacteria eventually penetrate into the blood stream and produce septicemia and overwhelming infection. If only a relatively few leukocytes emigrate at the infected site, the area will be effectively sterilized. In more recent experiments we have observed that even rough pneumococci, which are avirulent for normal rabbits because they are so readily phagocytosed, grow progressively in the tissues of rabbits deprived of leukocytes and that, though they remain rough and without capsule, they produce large hemorrhagic lesions typical of those caused by virulent pneumococci, with eventual septicemia and death. In man the various conditions pro-

44. Rous, P.: J. Exper. Med. **41**:399, 1925.

ducing severe leukopenia provide an analogous demonstration of the essential rôle of the phagocytes in defense against many types of bacteria, for whenever the number of leukocytes falls to a low level the bacteria that enter the tissues grow freely to colossal numbers and overwhelm the body. Subacute bacterial endocarditis is, likewise, of interest in demonstrating that in man, except in the several infections during which specific lysins are developed, specific acquired immunity is helpless without the cooperation of the phagocytes. Histologic study shows that most of the bacteria in the valvular vegetations, although intimately in contact with the antibody-containing plasma, are out of the reach of phagocytes and that they therefore proliferate there progressively, although the infection creates so high a degree of immunity that, in spite of the long continued septicemia, the bacteria practically never colonize and produce lesions in other tissues to which leukocytes have ready access, except when a mass of bacteria-laden vegetation is swept away from the valve and impacted in a distant blood vessel. Even in this event the resulting lesion is usually soon sterilized by the phagocytes of the attendant inflammation.

The experiments on leukopenic animals that I have mentioned demonstrated that acquired immunity creates no condition of the fixed tissues which can prevent the progressive and overwhelming growth of bacteria in the absence of the phagocytes, at least of the bacteria studied, and they failed to support the frequently encountered assumption that antibody may exhibit a direct bactericidal power in vivo which is not demonstrable in vitro. Tuttle and Cannon [45] have more recently brought forth evidence that bears, in a way, on the same point. They have shown that if bacteria are kept out of reach of phagocytes by implanting them in agar in the tissues, they grow freely at the very margin of the focus that is bathed in the fluids of the immune body.

Regarding the lymphocyte, I am sure that all who are engaged in the study and teaching of pathology will agree that the complete ignorance of the function of this cell is one of the most humiliating and disgraceful gaps in all medical knowledge. Produced daily in enormous numbers by a mass of distributed lymphoid tissue which, if gathered together, would form one of the most imposing organs of the body, normally constituting one fourth of the circulating leukocytes and increasing or decreasing markedly in number in various types of infection and in various other pathologic states, these cells must undoubtedly serve the body in a most essential way, and yet no information is possessed regarding their function apart from speculation, based on evidence that is equivocal, to say the least. It is still stated that lymphocytes are espe-

45. Tuttle, W. M., and Cannon, P. R.: Arch. Surg. **30**:243, 1935.

cially rich in lipase, though the most careful investigations have been unable to confirm this; that their presence protects the body against cancer, though it is known that cancer cells grow freely in lymph nodes; that lymphocytes are discharged into the intestine to aid in digestion or in detoxification, though no evidence has been brought forward to show that they play a rôle in either of these directions, and that they can be transformed into macrophages, granulocytes, plasma cells and even red blood cells, though convincing proof is still wanting. They can always be observed after a day or two in areas of inflammation, and when the inflammation is produced by bacteria or bacterial products they are often present in enormous numbers, but there is not the slightest notion of what they are doing there. They phagocytose neither bacteria nor other particulate matter. Congregated often in the more peripheral parts of the lesion, they have the appearance of phlegmatic spectators passively watching the turbulent activities of the phagocytes. Literally, nothing of importance is known regarding the potentialities of these cells other than that they move and that they reproduce themselves.

I have recently been interested in an observation that may possibly point toward at least one function of the lymphocyte. It has long been known that during the course of bacterial infection the spleen enlarges and that in its pulp there appear many mononuclear cells, with large vesicular nuclei and abundant basophilic cytoplasm. This is the condition designated as acute splenic tumor. Although not so universally recognized, it is no less easy to see that a precisely similar reaction occurs in the lymph nodes that drain infected areas. The nature and significance of the peculiar large cells in acute splenic tumor have been the subject of much speculation. Some investigators have regarded them as macrophages, others as lymphoblastic cells and perhaps the majority as young myeloid cells. Some have regarded the reaction as having for its purpose the production of phagocytes to help combat the bacteria; others have suggested that it may be associated with the formation of protective substances, and others, that it represents a reaction to toxic bacterial products.

Since both acute splenic tumor and formation of antibody are phenomena which occur together in all sorts of bacterial infection and since there is evidence which suggests that the spleen plays an important rôle in the formation of antibody, I carried out experiments last year in an attempt to determine whether acute splenic tumor would appear during the formation of antibody in the absence of bacteria or their products. Briefly, it was observed that all the essential characteristics of acute splenic tumor could readily be produced by injecting parenterally, by any route, foreign serum or other foreign protein, whether of

bacterial or of nonbacterial origin.[46] The specific cells arise by enlargement and by mitosis of cells of the malpighian bodies and pulp which resemble lymphocytes. Mrs. Lewis, Dr. Wintrobe and I have since been engaged in an attempt to determine the precise nature of the transformed cells. From vital and motion-picture studies of tissue cultures it is clear that the enlarged cells are not macrophages and that they are not phagocytic. Their manner of locomotion is entirely different from that of macrophages or granulocytes but is precisely that characteristic of lymphocytes. No relation has been noted between the development of these cells and the number of local or circulating granulocytes or macrophages. Whether they represent lymphoblasts or lymphocytes that are in a state of specific activity we are as yet unable to state.

The conditions under which these cells appear tempt one to wonder whether they may play a rôle in the formation of antibody. The production of antibody is widely believed to be accomplished by the macrophages, chiefly for two reasons: first, because antibody has been shown to appear earlier and in higher concentration in the spleen and lymph nodes under certain conditions than in other tissues in which macrophages are less abundant and, second, because it has seemed reasonable to believe that antibody could be formed by the cells which phagocytose bacteria and foreign colloids, and this the macrophages do. Attempts to solve the problem by blockade of the macrophage system have led to contradictory results and are subject to a variety of interpretations. I have always regarded the theory of the macrophage origin of antibody as the most probable, but it must be admitted that the argument of phagocytic activity would apply equally well to the polymorphonuclear neutrophil and also that the spleen and lymph nodes, in which antibody formation appears to be particularly abundant, contain many more lymphocytes than macrophages. It may be remarked that the large basophilic lymphoid cells occur not only in the spleen and regional lymph nodes of the infected body; they usually appear, often in great numbers, at the infected site as well. They were clearly described, for example, at the sites of vaccination in the interesting studies of Tuttle and Cannon [45] on local immunization. If the lymphocyte actually gives rise to the macrophage, as suggested by the studies of Bloom,[47] Parker and Rhoads [48] and others (a view which, however, is by no means generally accepted at present), and if the macrophage really produces antibody, this extraordinary lymphocytic response to foreign protein might be linked with the formation of antibody in that manner. We have, however, repeatedly watched these enlarged lymphoid cells

46. Rich, A. R.: Proc. Soc. Exper. Biol. & Med. **32**:1349, 1935.
47. Bloom, W.: Proc. Soc. Exper. Biol. & Med. **24**:567, 1927.
48. Parker, F., and Rhoads, C. P.: Am. J. Path. **4**:353, 1928.

divide into daughter cells of the same type, but so far we have obtained no evidence that they become transformed into either macrophages or granulocytes, whether in tissue cultures or within the body.

At present, only one thing is clear in regard to the significance of this reaction; it is the earliest morphologic reaction that we have been able to detect anywhere in the body after the introduction of foreign protein into the blood stream. It is highly developed long before any change in the number or appearance of the macrophages can be detected. Since it regularly results from the injection of foreign protein of any kind, it is difficult to escape the conviction that one function of the lymphocyte is concerned with the handling of protein that enters the tissues undigested. This view was expressed long ago by Kuczynski [49] as a result of his own very interesting studies. We have so far been unable to produce the reaction with nonantigenic carbohydrates or lipoids. It is obviously important to know whether nonprotein antigens incite the reaction, and this Dr. Felton and I are attempting to learn.

There are, of course, many other important problems relating to the aforementioned cells and to other cells of the inflammatory exudate, but I wish to consider the much discussed question of allergic inflammation.

For many years, largely as a result of studies on tuberculosis, it has been widely believed that the state of hypersensitivity is one of the chief mechanisms of acquired immunity—and this for the reason that one of the results of hypersensitivity is the occurrence of accentuated inflammation at any site at which bacteria lodge in the sensitized body. This exaggerated inflammation has been shown to be the result of the fact that bacterial substances which are relatively harmless for the normal body are violently irritating and necrotizing for hypersensitive tissues. The damaging and destructive effects of local and general hypersensitive reactions have always been recognized, but they have been regarded as necessary evils that, unfortunately, had to be borne for the protection of the body as a whole, for it has been assumed that the mechanical effect of the exaggerated allergic inflammation is responsible for the localization of bacteria in the immune body, and, in addition, the exaggerated inflammation has been assumed to be necessary for the more efficient destruction of bacteria, which is characteristic of the immune state.

While the arguments on which this belief was based are attractive and reasonable, I wish to stress the fact that the hypothesis that the hypersensitive state is necessary for the operation of acquired immunity has been widely accepted for years throughout the world in spite of the

49. Kuczynski, M. H.: Verhandl. d. deutsch. path. Gesellsch. **19**:87, 1923.

fact that not a single experiment has been placed on record in which hypersensitivity was shown to be necessary for protection at any stage in the development of acquired immunity, whether in tuberculosis or in any other infection. From time to time an occasional clinician has pointed out that his experience did not support the general teaching that hypersensitivity and immunity parallel each other, and from time to time investigators have recorded the same opinion, but even such suggestive observations as those of MacKenzie and Woo [50] failed to disturb the general belief in the necessity of hypersensitivity for immunity.

Several years ago a variety of observations that I have discussed in detail elsewhere [51] led us to question the validity of the hypothesis that hypersensitivity is necessary for immunity and to attempt to test it by pointed experiment. The result was that we found that there is no real basis for that widely accepted view. In a series of studies carried out in collaboration with Dr. A. M. Chesney, Dr. T. B. Turner, Dr. J. H. Brown and Dr. F. B. Jennings Jr., we demonstrated in a variety of ways and for a variety of infections in which immunity was assumed to be dependent on hypersensitivity that acquired immunity functions efficiently in the complete absence of allergic inflammation, whether in the face of small or of enormous doses of bacteria, and we could find no period or stage during the development of immunity in which allergic inflammation was necessary either for the localization or for the effective destruction of the bacteria.

First, we found that acquired immunity can be established without the concomitant development of hypersensitivity [52]—a fact indicated by the earlier experiments of MacKenzie and MacKenzie and Woo [50] and by the studies of Swift and Derick,[53] though in the last-mentioned experiments the bacteria were of so low a virulence for the normal test animals that no adequate study of acquired resistance could be made; next, we showed that the acquired immunity of an immune, hypersensitive animal can be transferred passively to a normal animal, without the concomitant transfer of hypersensitivity,[54] and, finally, we demonstrated that when the hypersensitivity of an immune animal is

50. MacKenzie, G. M.: J. Exper. Med. **41**:53, 1925. MacKenzie, G. M., and Woo, S. T.: ibid. **41**:65, 1925.

51. (*a*) Rich, A. R., and McCordock, H. A.: Bull. Johns Hopkins Hosp. **44**:273, 1929. (*b*) Rich, A. R.: Arch. Int. Med. **43**:691, 1929; (*c*) Bull. Johns Hopkins Hosp. **47**:189, 1930.

52. Rich, A. R.; Chesney, A. M., and Turner, T. B.: Bull. Johns Hopkins Hosp. **52**:179, 1933.

53. Swift, H. F., and Derick, C. L.: J. Exper. Med. **49**:883, 1929.

54. Rich, A. R., and Brown, J. H.: Proc. Soc. Exper. Biol. & Med. **27**:695, 1930.

abolished by desensitization and complete desensitization is maintained throughout the entire period of the infection, immunity remains intact.[55]

All these observations, which demonstrate that acquired immunity can operate in full force against millions of lethal doses of virulent bacteria in the complete absence of allergic inflammation, as measured by the histologic response not merely to the bacterial antigens but to the intact bacteria themselves, have since been confirmed by numerous investigators, both in this country and abroad, for all infections so far studied. The separation of acquired immunity from hypersensitivity has now been accomplished in pneumococcic, streptococcic and syphilitic infection and in infection with Pasteurella by ourselves and others.[56] That hypersensitivity is not necessary for the effective operation of acquired immunity in the particular infection for which the idea of the necessity

55. (*a*) Rich, A. R.; Jennings, F. B., Jr., and Downing, L. M.: Bull. Johns Hopkins Hosp. **53**:172, 1933. (*b*) In a recent review Dienes (Arch. Path. **21**:357, 1936), commenting on these experiments, stated that in his opinion bacterial allergy "represents a phase in the development of the immunity process, and it involves a much wider problem than the inflammatory reaction. . . . Desensitization depresses temporarily certain manifestations of the allergy, but one is not, therefore, justified in concluding that it suppresses the allergic condition itself." Our experiments were not concerned with a hypothetic, indefinable or undetectable state of the body but with the well recognized hypersensitivity of the tissues which, in the presence of the specific bacteria, provokes a visible and demonstrable tissue reaction of a definite character and measurable degree, and the capacity to react in this manner was abolished by our desensitizing procedure, leaving immunity to infection intact.

Dienes further stated that the method that we used in establishing hypersensitivity in our experiments on pneumococcic desensitization (i. e., repeated intracutaneous injections of killed pneumococci) would not, according to his observations, produce what he regarded as true bacterial allergy and that "no proof is presented that the inflammatory reaction obtained in these animals corresponded to the reaction to tuberculin." This method of producing hypersensitivity to the pneumococcus was first described by Avery and Julianelle (Proc. Soc. Exper. Biol. & Med. **26**:224, 1928) and has in our experience been highly effective. The same method has been successfully used by Swift and his associates (J. Exper. Med. **55**:591, 1932) in their studies on streptococcic hypersensitivity, and as for the efficacy of this method in producing hypersensitivity of the tuberculin type, I may state that hypersensitivity to tuberculin has been regularly established in human infants by Dr. Brailey, in this hospital, during the course of attempted prophylactic immunization by the intracutaneous injection of killed tubercle bacilli.

In expressing his opinion that our experiments do not demonstrate that active immunity can exist in the absence of bacterial allergy, Dienes apparently overlooked the fact that if, as he stated, he was unwilling to regard the type of hypersensitivity produced by intracutaneous vaccination as bacterial allergy, the experiments would still demonstrate that immunity is not dependent on the presence of bacterial allergy, even according to his own concept of the latter state, for the animals so treated always acquired a high degree of immunity to infection.

56. MacKenzie and MacKenzie and Woo.[50] Rich, Chesney and Turner.[52] Rich and Brown.[54] Rich, Jennings and Downing.[55a] Catron.[22] Clawson, B. J.: J. Infect. Dis. **53**:157, 1933.

of allergic inflammation was most deeply entrenched—namely, tuberculosis—has now been demonstrated by Rothschild and his co-workers [57] in our laboratory. Their painstaking and carefully controlled experiments, which showed that acquired immunity in tuberculosis remains intact in the face of complete and constantly maintained desensitization, have since been confirmed by Boquet,[58] Siegl [59] and Cummings and Delahant,[60] and Branch and Enders,[61] Branch and Cuff [62] and Clawson [63] have succeeded in producing immunity without hypersensitivity in tuberculosis by experiments of a different type. On the other hand, we showed several years ago that allergic inflammation which develops about bacteria after they reach the tissues is in itself totally incapable of fixing virulent bacteria locally or of protecting against fatal infection in the absence of specific immunity [51c]—an observation confirmed by Thomsen and Pedersen-Bjergaard,[64] Klopstock and his associates [65] and others. MacKenzie and Woo,[50] Julianelle,[66] Seibert [67] and Sabin and her associates [68] have shown that specific hypersensitivity to the bacterial protein in pneumococcic and tuberculous infection can be established without the concomitant development of immunity to the specific bacterium. In none of the latter studies, however, was it stated whether or not the injection of living bacteria elicited allergic inflammation in the hypersensitive animals, and in the absence of that information the experiments, though highly interesting, cannot fairly be regarded as demonstrating that the body can respond to bacteria with specific, local hypersensitive inflammation and yet show no immunity.[69]

57. Rothschild, H.; Friedenwald, J. S., and Bernstein, C.: Bull. Johns Hopkins Hosp. **54**:232, 1934; Tr. Nat. Tuberc. A. 1931, p. 149.

58. Boquet, A.: Compt. rend. Soc. de biol. **112**:1168, 1933.

59. Siegl, J.: Beitr. z. Klin. d. Tuberk. **84**:311, 1934.

60. Cummings, D. E., and Delahant, A. B.: Tr. Nat. Tuberc. A., 1934, p. 123.

61. Branch, A., and Enders, J. F.: Am. Rev. Tuberc. **32**:595. 1935.

62. Branch, A., and Cuff, J. R.: J. Infect. Dis. **47**:151, 1930.

63. Clawson, B. J.: Proc. Soc. Exper. Biol. & Med. **31**:165, 1933.

64. Thomsen, O., and Pedersen-Bjergaard, K.: Acta path. et microbiol. Scandinav., supp. 16, 1933, p. 521.

65. Klopstock, A.; Pagel, W., and Guggenheim, A.: Klin. Wchnschr. **11**:1826, 1932.

66. Julianelle, L. A.: J. Exper. Med. **51**:625, 1930.

67. Seibert, F. B.: Proc. Soc. Exper. Biol. & Med. **30**:1274, 1933.

68. Smithburn, M. C.; Sabin, F. R., and Geiger, J. T.: Am. Rev. Tuberc. **29**:562, 1934.

69. In only one of the four studies last mentioned (that of Seibert [67]) was the test for immunity carried out under conditions suitable for the observation of the local reaction to infection. In reply to a request for information regarding the point in question, Dr. Seibert recently informed me that the animals sensitized to tuberculin protein did not react with allergic inflammation to the intracutaneous

(Footnote continued on next page)

In all our experiments in which hypersensitivity was removed from the picture by one or another of the methods already mentioned, both the localization of the bacteria and their destruction were as efficient as they were in the hypersensitive animals used as controls, in which much more inflammation and far more damage to the tissue occurred. I have pointed out in an earlier part of this paper that the specific localization of bacteria in the body with acquired immunity is effected primarily by a mechanism that is independent of the mechanical effects of inflammation. As for the destruction of the bacteria, the most impressive characteristic of acquired immunity in all our experiments in which hypersensitivity was eliminated was the small amount of inflammation that sufficed to rid the immunized body of bacteria on reinfection. In the immune animals in which hypersensitivity was eliminated, the amount of inflammation was always much less than that in either the nonimmune or the hypersensitive animals; and in our experiments on leukopenic immunized animals already mentioned we were repeatedly impressed by the fact that, whereas no degree of immunization could prevent the progressive growth of the bacteria in the complete absence of the leukocytes, if only a relatively small number of leukocytes appeared at the infected site—a minute fraction of the number emigrating in hypersensitive animals possessing a full quota of leukocytes—the bacteria were always readily overcome. In this connection I wish particularly to emphasize that in the hypersensitive body not only the tissue cells but the phagocytes themselves are far more susceptible to the damaging effects of the bacterial antigens than are the phagocytes of the nonhypersensitive body. This fact was demonstrated in tissue culture studies on the hypersensitivity of phagocytes, carried out in collaboration with Mrs. Lewis [70] and confirmed by Aronson,[71] and also by observations in vivo of Stewart, Long and Bradley.[72] This is obviously a point of importance in any consideration of the defensive potentialities of allergic inflammation, for a given number of phagocytes that are not hypersensitive should be more efficient than a far larger number that can be damaged or killed by reason of their hypersensitivity.

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injection of living tubercle bacilli. Whether this indicates that in the absence of immunity there was not sufficient destruction of bacilli to liberate bacterial substances in an amount capable of inciting a hypersensitive reaction in the sensitized body, whether sensitization to the whole bacillus is different from sensitization to tuberculin protein or whether the sensitization established was not of a degree sufficient to produce a reaction with the dose of bacilli injected cannot be decided on the basis of the available information.

70. Rich, A. R., and Lewis, M. R.: Bull. Johns Hopkins Hosp. **50**:115, 1932.

71. Aronson, J. D.: J. Exper. Med. **54**:387, 1931.

72. Stewart, F. W.; Long, P. H., and Bradley, J. H.: Am. J. Path. **2**:47, 1926.

It is clear from the study of the tissues in experiments of all these types that, particularly through the immobilizing and opsonizing effects of antibody, acquired immunity enhances so greatly the protective power of the inflammatory exudate that an astonishingly small amount of inflammation suffices to sterilize the tissues rapidly and effectively. Indeed, as I have pointed out elsewhere,[73] this is the state of affairs that obtains in natural immunity. The body that is naturally immune to a particular bacterium does not respond to the presence of that bacterium with exaggerated inflammation and destruction of tissue. On the contrary, it requires only a slight amount of inflammation to sterilize the site of infection, and destruction of tissue is negligible. It is interesting that the doctrine that exaggerated inflammation is necessary for protection has, in the past, been extended even to natural immunity, and it has been suggested[74] that the degree of natural immunity of different animal species depends on the intensity of inflammation with which the species responds to the bacterium in question. The careful studies of Long, Holley and Vorwald and of Vorwald[75] have shown that this is not the case. Quite on the contrary, they found (and their observations are easy to confirm) that the degree of natural immunity is inversely proportional to the amount of inflammation with which the body responds to the bacterium in question.

What, finally, is the purpose of this hypersensitive state that renders relatively bland bacterial and other foreign antigens so highly toxic for the sensitized tissues and causes not only destruction of allergic tissue and deleterious constitutional effects in association with bacterial infection but also, under different conditions, anaphylaxis, asthma, hay fever, food sensitivity and serum sickness? That the potentiality for the development of this remarkable state serves a useful purpose in some direction seems altogether likely, though up to the present no satisfactory demonstration of its usefulness has been made. In conditions such as asthma and hay fever the virtue of abolishing hypersensitivity whenever possible has been learned. In view of the studies already described, which have yielded abundant proof that hypersensitivity is not necessary for the operation of acquired immunity against bacteria, an attitude of resignation toward the deleterious effects of hypersensitivity in association with bacterial infection need no longer be adopted. It is highly desirable to search for safer and more efficient methods of desensitization than are now possessed for use in appropriate conditions in man—not only in tuberculosis but in a variety of other infections in which hypersensitivity exerts damaging effects—with the knowledge that if the body

73. Rich, A. R.: Lancet **2**:521, 1933.

74. Krause, A. K.: Am. Rev. Tuberc. **6**:233, 1922.

75. Long, E. R.; Holley, S. W., and Vorwald, A. J.: Am. J. Path. **9**:329, 1933. Vorwald, A. J.: Am. Rev. Tuberc. **27**:270, 1933.

can be freed from the dangers of the hypersensitive state it will not at the same time be robbed of the protection of its acquired resistance. Furthermore, the possession of an adequate method of desensitization would soon serve to instruct the investigator as to whether there may be any conditions in man under which hypersensitivity plays a beneficial rôle in bacterial infection. Although no such condition has yet been demonstrated, the studies that I have described do not exclude the possibility that it may exist. Meanwhile, in the light of the facts presented in this paper, it is at least reasonable to suggest that one should impartially refrain from attributing to hypersensitivity any particular protective effect observed in the immune body until a test has been carried out to determine whether or not that protective effect occurs in the immune body in the absence of hypersensitivity, for up to the present each time that the matter has been put to a crucial test, from whatever angle, it has been found that acquired immunity is not dependent on the occurrence of allergic inflammation.

Notes and News

New Cancer Hospital.—It is reported that the General Education Board has given $3,000,000 to the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, for a new hospital building of two hundred beds. Construction will start next autumn. The Memorial Hospital, which was founded in 1884, is considered to be the oldest special cancer hospital in this country; it has been affiliated with Cornell University Medical College since 1913.

Grants in Aid of Research.—The National Research Council announces that a limited fund is available for grants in aid of medical research. Applications should be submitted to the secretary of the division of medical sciences of the council, 2101 Constitution Avenue, Washington, D. C., on or before October 1 next in order to be considered in November.

Plans for Institute of Forensic Medicine.—It is reported that the establishment of an institute of forensic medicine in New York City by the cooperation of the medical examiner's office and the medical schools of the city is under consideration.

Society News.—The tenth congress of the International Union Against Tuberculosis will be held in Lisbon, Portugal, on Sept. 7 to 10, 1936. Information in regard to membership and other matters may be obtained from the National Tuberculosis Association, 50 West Fiftieth Street, New York.

Esmond R. Long has been elected president of the American Tuberculosis Association.

The Biologic Photographic Association will hold its sixth annual meeting in Boston on Sept. 24 to 26, 1936. The secretary is Miss Anne Shiras, Elizabeth Steel Magee Hospital, Pittsburgh.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Compensatory Changes in the Remaining Lung Following Total Pneumonectomy. W. F. Rienhoff Jr., F. L. Reichert and G. J. Heuer, Bull. Johns Hopkins Hosp. **57**:373, 1935.

Following total pneumonectomy in dogs the compensatory changes in the remaining lung consist in simple dilatation of the respiratory lobules or the definitive respiratory units made up of the respiratory bronchioles, the alveolar ducts, the atria, the alveolar saccules and the alveoli. This dilatation comes in response to increased physiologic demands and is compensatory. It is in no sense of the word an emphysema, and there is no interruption or diminution of the elastic tissue or fusion of the alveoli to suggest pathologic change in the lung parenchyma. There is no increase in the number of the bronchial trees or change in their pattern, and apparently the blood vascular system is unaffected except for possible dilatation. No evidence of true hyperplasia or hypertrophy is found. The lack of uniformity of the dilatation of the alveoli in any section was due to the fact that the serial sections cut through the alveoli at different levels of the block of tissue. The maximum diameters of different alveoli are, of course, situated in different planes.

From the Authors' Conclusions.

Studies on Inflammation. V. Menkin, J. Infect. Dis. **58**:81, 1936.

If a culture of Staphylococcus aureus, aleuronat or turpentine is inoculated into the skin of a rabbit in an area where virulent type III pneumococci have been injected the rabbit shows an increase in survival time. The increase in resistance thus manifested is due to a delay in the spread of the pneumococci from the site, and this is referable in turn to early mechanical obstruction by thrombosis of lymphatics and by coagulation of plasma in the inflamed area. The presence of such a barrier has been correlated with the inability of a dye to diffuse readily into the regional lymphatics from the site of inflammation and with the results of studies on the presence of pneumococci in the blood stream.

The observations indicate that the rapidity of invasion by a micro-organism as modified by the local inflammatory reaction is a factor in determining the resistance or immunity of the host, but that induced alterations in the rate of dissemination may leave the virulence of the bacterium essentially unaffected.

From the Author's Conclusions.

Experimental Anemia in Monkeys. L. Wills and A. Stewart, Brit. J. Exper. Path. **16**:444, 1935.

The hematopoietic system of the rhesus monkey behaves remarkably like that of man, and therefore the animal is suitable for experimental study of the different forms of anemia, particularly the macrocytic. The only finding wherein the picture of macrocytic anemia in the monkey differs from that of human anemia is the hypertrophy of the bone marrow. The megaloblastic reaction was very similar to that seen in pernicious and other types of human macrocytic anemia, but the large pale primitive cells which were present in such large numbers in the marrow from some of the animals are not commonly duplicated in preparations from human material. These cells were found in greatest number in the bone marrow from

a monkey that died during a blood crisis during which very numerous nucleated red cells appeared in the blood stream and they appear to have been stem cells, though their relation to the red and white cell series could not be determined from the preparations studied.

FROM THE AUTHORS' DISCUSSION.

EFFECT OF MUSTARD GAS ON THE SKIN. J. DÖRFFEL and PÖPPING, Virchows Arch. f. path. Anat. **295**:1, 1935.

The effects of mustard gas were studied in the skin of rabbits and young pigs. In the course of the experiments the authors had occasion to observe the effects of the poison on their own skin. Following contact of the gas with the skin the reaction was latent for seven hours. Then the skin became hyperemic and painful and itched intensely. Pain and itching were constant features thereafter, interfering with well-being and persisting even after healing of the lesion. At twelve hours a ring of vesicles began to form at the periphery of the lesion. The vesicles were fully developed at seventeen hours and broke down after the second day. The blister fluid was sterile. It caused no reaction when applied to a fresh area of skin. By the sixth day the entire lesion was ulcerated. It persisted as a sluggish necrotic ulcer until the third to fourth week, when healing slowly began and was completed by the sixth week. In animals the irritant caused local hyperemia and edema, followed by vesication, leukocytic infiltration, necrosis, ulceration and slow healing. The lesion in man and in animals is likened to a third degree roentgen ray burn.

O. T. SCHULTZ.

Pathologic Anatomy

CONGENITAL LYMPHANGIECTASIS (LYMPHEDEMA). P. B. MASON and E. V. ALLEN, Am. J. Dis. Child. **50**:945, 1935.

The microscopic appearance of congenital lymphedema is entirely characteristic and need not be confused with other types of lymphedema. Unfortunately, the characteristics of the tissue which were observed microscopically in five cases do not adequately explain the occurrence of congenital lymphedema. This condition is characterized by the replacement of normal subcutaneous fat with widely dilated lymphatic spaces and with fibrous tissue, but the cause of these changes is obscure. It is probable that the original abnormality is the increased surface area which is occupied by lymphatic vessels. Such a change would produce stasis of lymph, which may cause fibrosis, as lymph is an excellent culture medium for the growth of fibroblasts. What causes the lymphangiectasis? It may be viewed as a developmental anomaly, which is subject to the same questions about origin as are other kinds of developmental anomalies. It seems certain that congenital lymphedema is not simply a condition which develops during intra-uterine life and which can be compared with lymphedema that begins at the age of 3 years. The microscopic appearance of the tissue is so characteristic that the term "congenital lymphangiectasis" should be used to replace the term "congenital lymphedema," because "congenital lymphangiectasis" is a better descriptive term.

FROM THE AUTHORS' SUMMARY.

PERSISTENT TRUNCUS ARTERIOSUS COMMUNIS. ALLAN ROSS, Am. J. Dis. Child. **50**:966, 1935.

An example of almost complete persistence of the truncus arteriosus communis is reported. Associated with this were a rudimentary aorticopulmonary septum, a small pulmonic trunk with normal right and left branches, an aortic arch on the right side, the absence of an innominate artery, a four-cusped semilunar valve indicating a detorsion defect of under 45 degrees, a defect in the base of the interventricular septum, a patent foramen ovale and an irregular thickening of the cusps of the valve. There are two fundamental arrests and aberrations which are

responsible for this malformation. The first is a failure of the aorticopulmonary septum to form. This must depend ultimately on defective formation of spurs and on caudal migration. The second basic factor is faulty torsion, directly related to which is a defect in the upper portion of the interventricular septum. It is certain that these abnormal events occur in early fetal life, when the embryo has attained a length of from 7 to 8 mm. It is of little help to invoke "a diffuse weakening in the growth vitality of various cell groups." One must confess complete ignorance of the reasons for the arrest in formation of spurs and the aberration in torsion.

FROM THE AUTHOR'S SUMMARY.

LESIONS OF THE LEFT AURICLE IN RHEUMATIC FEVER. L. GROSS, Am. J. Path. **11**:711, 1935.

Gross and histologic observations on the left auricle, based on an examination of eighty-seven rheumatic hearts, are described. The material is classified into five clinical groups depending on the course of the disease. It is shown that macroscopic lesions of the left auricle occur in 80 per cent of the cases and microscopic lesions in 100 per cent. In the acute cases the lesions are significant and characteristic. In the chronic cases they are considerably milder and often difficult to differentiate from normally occurring histologic changes. A description is also given of the changes according to age periods, as observed in fifty hearts.

FROM THE AUTHOR'S SUMMARY.

ANOMALIES OF THE CIRCLE OF WILLIS WITH RESULTING ENCEPHALOMALACIA AND CEREBRAL HEMORRHAGE. O. SAPHIR, Am. J. Path. **11**:775, 1935.

Anomalies of the circle of Willis, with resulting interruption of the circulation between the internal carotid and vertebral arteries, may form the anatomic basis of cerebral vascular disturbances. The recognition of such anomalies is important because they aid in the explanation of cerebral hemorrhage and encephalomalacia on morphologically demonstrable grounds in the absence of occluding lesions of the supplying arteries. In addition to local causes for encephalomalacia and cerebral hemorrhage one must consider the condition of the myocardium and evidence of myocardial failure in the various organs. Three brains are described which revealed anomalies of the circle of Willis involving the posterior communicating arteries and in one case an abnormal origin of the posterior cerebral artery. Two of these brains revealed areas of encephalomalacia and cerebral hemorrhage without the presence of occluding lesions in the supplying arteries. Whereas the posterior communicating arteries are not essential in the maintenance of the circulation of the brain under normal conditions, an unhampered collateral anastomosis between the internal carotid and vertebral arteries is important in instances of diffuse arteriosclerosis of the arteries of the base of the brain combined with beginning myocardial failure. This conception of the origin of these lesions of the brain is based entirely on morphologically demonstrable changes and does not require the assumption of theoretical functional disturbances of the circulation. Perhaps similar anatomic observations may explain anatomic changes elsewhere which now are attributed to functional disturbances.

FROM THE AUTHOR'S SUMMARY.

SYMMETRICAL TRAUMATIC FRACTURES OF THE CRANIUM: SYMMETRICAL FRAGMENTATION; COMMENTS ON THEIR MECHANISM. E. R. LECOUNT and JACK HOCKZEMA, Arch. Surg. **29**:171, 1935.

Recent traumatic fractures of the cranium produced by blunt force in 1,278 bodies are analyzed. All except 100 occurred in adults more than 20 years of age. Long radiating fractures are numerous as compared with the findings some years ago, when comminuted and depressed localized fractures were common. This has resulted from the increase of motor car accidents. Eighty of the 1,278 fractures

are distributed equally on the right and left sides of the cranium or, coursing in the sagittal midline, have broken the skull into lateral halves. Forty-one of the 80 are ring-shaped fractures about the foramen magnum, mainly in the posterior fossa or spread so as to include the posterior fossa and portions of the middle fossa and the vault. The authors record an extensive historical analysis of the relationship of the spine and the weight of the bodies and extremities to ring fractures about the foramen magnum. This brief of the literature includes reports of 45 more or less complete ring fractures of the cranium. About half resulted from headlong falls in which the head was struck first. Among the 80 symmetrical fractures in the authors' series, there are 5 ring fractures of the vault and 36 of the base. The large ring fracture in each case (20) has split from the base a corresponding concave disk, mainly of occipital bone, the center near or opposite the top of the spine. There are some variations. The transition between the large and small (16) ring fractures is gradual. Important clinical information, details of the postmortem examinations and illustrative sketches are given for the 80 fractures of the cranium. According to published accounts, ventral ring fractures of the cranium have received scant attention. The authors analyze 5, three fairly symmetrical, 2 not. Other fractures described are: 6 symmetrical fractures of the orbital roofs, 10 dorsal transverse, 6 ventral transverse and 22 sagittal fractures. In general, one of the main justifiable conclusions, the authors state, is that the direction of the force and the fracture are usually parallel. From the place struck squarely with blunt force, fractures should radiate in all directions as meridians toward opposite poles. All such meridians lie in planes along which violence is propelled. The authors conclude their report in a discussion of the anatomic structure of the cranium and the importance of its buttresses. E. F. Hirsch.

Jaundice Produced by a Diverticulum of the Duodenum. W. M. Nicholson, Bull. Johns Hopkins Hosp. **56**:305, 1935.

A case of duodenal diverticulum is reported in which intermittent jaundice was caused by compression of the common bile duct by the anomaly. Attention is called to similar cases in the literature. Roentgen studies of the gastro-intestinal tract are urged in all cases in which there is an obscure jaundice.

From the Author's Summary.

Relation of the Cushing Syndrome to the Pars Intermedia of the Hypophysis. W. G. MacCallum, T. B. Futcher, G. L. Duff and R. Ellsworth, Bull. Johns Hopkins Hosp. **56**:350, 1935.

A typical case of the Cushing syndrome is described with symptoms and anatomic changes corresponding with those already known. The cells of the tumor are cylindric and arranged radially about capillary blood vessels. In their form these cells resemble those of the pars intermedia and are different in form and arrangement from the basophil cells of the anterior lobe. Furthermore, on staining by a method described by Cowdry with copper acetate and hematoxylin it is found that, while the basophils of the anterior lobe are stained black, the cells of the tumor, like those of the pars intermedia, including those which radiate into the posterior lobe, remain unstained. For these reasons and because of its continuity with that tissue, it is concluded that the tumor is derived from the cells of the pars intermedia. If this is true, the remarkable disturbances of function which accompany the presence of this tumor, most of which have been thought dependent on some change in the anterior or the posterior lobe, may rouse new interest in the activities of the pars intermedia. From the Authors' Conclusions.

The Plasma Phosphatase in the Various Types of Jaundice. F. K. Herbert, Brit. J. Exper. Path. **16**:365, 1935.

By using the method of Jenner and Kay for the estimation of phosphatase it has been found that in jaundice due to gross mechanical obstruction of the bile

ducts the phosphatase of the plasma is always above normal and usually is markedly raised. In jaundice due to toxic or infective disease of the liver the phosphatase of the plasma is in many cases normal and in others usually only slightly raised, though occasionally it is high. In hemolytic jaundice the phosphatase of the plasma is normal or shows no significant increase. The diagnostic value of the test is limited by the fact that moderate rises in the phosphatase of the plasma may occur both in jaundice due to mechanical obstruction and in toxic or infective jaundice. The estimation of phosphatase may give assistance in diagnosis in some cases. The figures for phosphatase and directly reacting bilirubin in the plasma do not run parallel. It may be possible, however, to explain the retention of phosphatase as due to biliary obstruction without any alteration in the accepted interpretation of the van den Bergh test. FROM THE AUTHOR'S SUMMARY.

THE EFFECT OF INJURY ON THE LEVEL OF THE PLASMA PROTEINS. D. P. CUTHBERTSON and S. L. TOMPSETT, Brit. J. Exper. Path. **16**:471, 1935.

Trauma such as to cause fracture of one or more of the long bones, or an effusion into a knee joint or a surgical procedure such as the excision of a knee joint causes an immediate marked disturbance in the total amount and relative proportions of the different proteins in the plasma as fractioned by neutral salt precipitation. The normal level is slowly regained. The general effect is a slight fall in the albumin moiety coupled with a marked rise in the globulin fraction. Fibrinogen is often appreciably raised. FROM THE AUTHORS' SUMMARY.

EXAMINATION OF URINARY AND BILIARY CONCREMENTS BY THE USE OF ULTRAVIOLET RAYS. V. FABER, Frankfurt. Ztschr. f. Path. **47**:421, 1935.

Faber used the mercury vapor arc lamp fitted with the "uviol dark glass filter" to investigate the fluorescent properties of urinary and biliary concrements. Many more layers and more detailed structures were observed than could be seen by the use of normal light. The exact chemical components, however, could not be determined by this method because of the discoloration of the concrements by bile pigment.

OTTO SAPHIR.

THE COPPER CONTENT OF INTERNAL ORGANS IN CERTAIN SELECTED CASES. W. GERLACH, Virchows Arch. f. path. Anat. **295**:394, 1935.

To his previous systematic quantitative spectrographic analyses of internal organs for copper Gerlach adds five because of the deviation of the findings from those reported previously. Four related to children. One with mongolian idiocy and one with congenital atresia of the hepatic duct and congenital biliary cirrhosis had lower than average amounts of copper in the liver. In one with icterus gravis the quantity of copper in the liver was within the normal range, indicating that icterus of itself need have no effect on hepatic copper. In an 11 year old boy with atrophic cirrhosis hepatic copper was high, as had been found to be the rule in adults with atrophic cirrhosis. An exception to this rule was indicated in the fifth analysis—that of liver from an adult with atrophic cirrhosis: The copper content of the liver was normal.

O. T. SCHULTZ.

Microbiology and Parasitology

THE COLONY MORPHOLOGY OF TUBERCLE BACILLI. K. C. SMITHBURN, J. Exper. Med. **62**:645, 1935.

All the strains of human tubercle bacilli described in this report as recently isolated from clinical cases proved to be virulent and showed but slight differences in pathogenic properties. Nor did they show extreme variation in virulence when

compared with strain H-37, isolated many years ago. Between twelve bovine strains cultivated in vitro for varying periods there was a much wider range in virulence, some being so attenuated as to give regressive lesions in both rabbits and guinea-pigs, while other strains were highly pathogenic. In general, the more recently isolated strains were the more virulent. Attenuation is apparently not the same for all strains. Studies of cultures recovered from animals into which they had been inoculated demonstrated that in the case of bovine tubercle bacilli virulence is correlated with three phenomena: the number of bacilli which can be stained in the tissues of these animals, the number of organisms recoverable in cultures from the tissues and the proportion of smooth colonies in these cultures. All cultures of either human or bovine type recovered from animals showed either two or three types of colonies. In general, the percentage of the smooth form varied directly with virulence. However, some smooth colonies were present in strains having little or no pathogenicity. One example was cited of an avian strain which had lost its virulence while retaining the smooth colony—indicating that, although pathogenicity is usually correlated with the smooth colony, it is not necessarily so. And smooth variants devoid of virulence occur. In the comparison of the effects of the same human strain on rabbits and guinea-pigs, it was shown that native resistance (of the rabbit) was associated with a power to dissociate the inoculated bacilli into a greater portion of rough forms and then to destroy them. This power is not possessed by the naturally susceptible animal (guinea-pig).

FROM THE AUTHOR'S SUMMARY.

BACTERIAL NUTRITION. S. A. KOSER and OTHERS, J. Infect. Dis. **58**:121, 1936.

A growth-stimulating factor which permits development of some of the more fastidious bacteria in a synthetic medium was found to be widely distributed in animal and plant tissues. Of the various sources tested, calf spleen, calf liver and yeast were the richest, although many tissues gave good results. The qualitatively similar responses of the organisms indicate marked similarity in the growth factors secured from widely different sources. FROM THE AUTHORS' SUMMARY.

THE LIFE-CYCLE OF THE PLEUROPNEUMONIA VIRUS. K. B. MERLING-EISENBERG, Brit. J. Exper. Path. **16**:411, 1935.

A well defined life cycle is described and illustrated for the pleuropneumonic virus in culture.

FROM THE AUTHOR'S SUMMARY.

THE CAUSAL AGENT OF BOVINE PLEUROPNEUMONIA. F. F. TANG, H. WEI and J. EDGAR, J. Path. & Bact. **42**:45, 1936.

Two rare morphologic elements of the organism causing bovine pleuropneumonia are described, namely, the ameboid and the giant ring form. Microscopic colonies have been demonstrated to occur in early serum broth cultures of the freshly isolated but not of the old laboratory strains of the organism. This confirms the authors' view that the mode of multiplication in the former is different from that in the latter. Filamentous forms of the organism, which were poorly stained and invisible when examined in the ordinary way, were distinctly seen when dark-field illumination was used. The organism was found to be resistant to ultraviolet rays and to the photodynamic action of methylthionine chloride (methylene blue). Its activity in steers may be enhanced by testicular extract of rats. The organism has been cultivated in the chorio-allantoic membrane of the chick embryo. No inclusion bodies were found, but the organism could be reisolated. Pseudopleuropneumonic forms have been found in the chick embryo and in the blood of the hen, guinea-pig, rabbit, goat, sheep, horse and man. They are probably formed from the protoplasm of red blood corpuscles by some process of protein dispersion.

FROM THE AUTHORS' SUMMARY.

DISSOCIATION OF THE TUBERCLE BACILLUS. KONRAD E. BIRKHAUG, Ann. Inst. Pasteur **54**:19, 1935.

Avian, bovine and human strains of tubercle bacilli all dissociate into three morphologically distinct types, i. e., rough (R), smooth (S) and chromogenic (Ch). The S variety predominates in avian strains and the R in mammalian strains. Of the three types, the chromogenic appears to be the most stable, but all three forms have been obtained from cultures of a single bacillus. Dissociation is carried out most successfully in a liquid medium, the cultures being left at room temperature after a short period of incubation at 37 C. Addition of anti-R or anti-S rabbit serum or of tissues from immune animals does not influence the dissociation.

The most distinct differentiation between the S and Ch and the R types is the growth in a liquid glycerin medium. The S and Ch types grow in two phases: At first there is a diffuse growth throughout the medium; this is followed by a clearing of the medium and the formation of a smooth glistening film. The R form presents a wrinkled, irregular and friable film of growth, without any diffuse growth. In liquid mediums without glycerin, the Ch and S types form no film. Incubation at 37 C. is essential for growth of the R type, while the others grow well at room temperature. Growth does not develop in anaerobic cultures. The optimal p_H for the Ch and S varieties is between 4 and 9. The film is formed, however, only between p_H 5.5 and 8, this being the optimal range for growth of the R type. In cultures of avian strains, the R variant shows an acid reaction, the S and Ch an alkaline reaction, after growth for ten weeks at 37 C. In cultures of mammalian strains, this process is reversed, the R culture being alkaline and the S and Ch acid. Mixed cultures of R and S types of avian strains produce a final alkaline reaction because of the rapid and dominant growth of the S variety. In mixed cultures of mammalian strains, an acid reaction is produced, the S type again being dominant. This antagonism between types in vitro appears to be the result of the presence of some stabilizing bacterial hormone in the S variety which transforms the type. Sugar fermentation reactions are practically identical for all types. In all strains the R variant is more resistant to dyes and disinfectants than the S and Ch types, but is less adaptable to the action of heat, light or conditions of unfavorable nutrition. The electrical potential of S types does not change with the age of the culture. However, in R forms it varies with the origin as well as with the age of the culture. In buffer solutions, the S variant of bovine strains agglutinates at a p_H of from 2.4 to 3; the R type, at from 4.2 to 4.8. The S variant of avian strains agglutinates at a p_H of from 3.6 to 4; the R, at from 6.8 to 7.2. The difference in p_H range is sharp in both avian and mammalian strains. In cellular morphology the Ch, R and S varieties of both avian and mammalian strains are not distinguishable from one another. In general, bacilli from R variants are somewhat longer, more slender and more granular than Ch and S types. The Ch and S variants of both mammalian and avian strains are less resistant to acid than are the R. The Ch and S varieties grow much more profusely (two to five times) than the R. Chromogenic variants of mammalian and avian strains are usually avirulent for experimental animals. If the bacilli are injected intravenously in strong doses, the animals occasionally die from a nonfollicular generalized bacillosis. The S types of avian strains are virulent for rabbits and hens but only slightly virulent for guinea-pigs, while the R variants are relatively avirulent for experimental animals except when injected intravenously in large doses. They then cause a form of chronic miliary tuberculosis. The S variants of mammalian strains are relatively virulent for rabbits and guinea-pigs when obtained from the first or second subcultures after dissociation. This virulence disappears rapidly, and injection (intravenous or interperitoneal) of large doses causes a generalized bacillosis from which the animal usually recovers. Subcutaneous injections cause a localized lesion which heals completely. Human strains of the R type are virulent for guinea-pigs, and bovine strains of the R type are virulent for both guinea-pigs and rabbits. The R type of attenuated BCG is an exception and is avirulent.

FROM THE AUTHOR'S SUMMARY.

INAPPARENT HUMAN TYPHUS. PAUL GIROUD, Arch. Inst. Pasteur de Tunis **25**: 74, 1936.

A study in various regions of Tunis where typhus fever is found indicated that inapparent typhus exists. This showing was based on Weil-Felix reactions. Of 116 persons who were ill but who were in contact with patients suffering from typhus, 5 had definite titers and several had notable titers. By inoculation of animals a virus was recovered from 3 of 27 persons thus tested. It is considered probable that infections are more common than the Weil-Felix reactions indicate, since inapparent infection does not necessarily induce marked agglutinative power.

M. S. MARSHALL.

ACUTE MILIARY TUBERCULOSIS FOLLOWING CURETTAGE IN A PATIENT WITH TUBERCULOUS ENDOMETRITIS. W. BÜNGELER, Frankfurt. Ztschr. f. Path. **47**:313, 1935.

The uterus of a 20 year old woman was curetted because of an abortion in the fourth month of pregnancy. The scrapings were not available for histologic examination. Four weeks later she showed signs of sepsis and died eight weeks after curettage. Autopsy revealed tuberculous salpingitis, tuberculosis of the cervix and uterus, generalized miliary tuberculosis and tuberculous meningitis. Büngeler stresses the danger of generalized tuberculosis from curettage in a patient with tuberculous endometritis.

OTTO SAPHIR.

Immunology

THE RELATIONSHIP OF CIRCULATING ANTIBODY TO THE LOCAL INFLAMMATORY REACTION TO ANTIGEN (THE ARTHUS PHENOMENON). J. T. CULBERTSON, J. Immunol. **29**:29, 1935.

Exact quantitative determinations of precipitin by means of Culbertson's so-called neutralization method showed a direct correlation between the titer of the precipitin in the serum of a rabbit and the severity of the Arthus phenomenon in its skin. The presence of less than 0.5 mg. of antibody protein per centimeter of the serum was associated with a mild Arthus phenomenon. The reaction was one of increasing severity as the quantity of the antibody surpassed from 0.75 to 1 mg. of protein per cubic centimeter of serum. The removal of the circulating precipitin by injecting antigen intravenously caused absence of the skin reaction in animals that had reacted positively. Experiments that established a general and a local passive transfer of the Arthus reaction and the so-called reversed Arthus reaction also confirmed the observation that circulating precipitin is necessary for the appearance of the skin reaction. Culbertson concludes that his results cast doubt on the conclusions of Kahn in the latter's numerous recent publications.

I. DAVIDSOHN.

THE FAILURE OF CONCENTRATED DIPHTHERIA ANTITOXIN TO GIVE FLOCCULATION WITH TOXIN. H. EAGLE, J. Immunol. **29**:41, 1935.

Eagle has demonstrated that the failure of concentrated solutions of pseudoglobulin prepared from diphtheria antitoxin to give flocculation with the toxin is not due to an absence of a union of the two in vitro. He added antihorse serum from rabbits to mixtures of diphtheria toxin with concentrated antitoxin. The supernatant fluid was not toxic. Proper control experiments that indicated the correct interpretation of the results are recorded.

I. DAVIDSOHN.

THE RELATIONSHIP BETWEEN COMPLEMENT AND PROTHROMBIN. A. J. QUICK, J. Immunol. **29**:87, 1935.

The common features of complement and prothrombin are: (*a*) the lack of species specificity; (*b*) the thermolability; (*c*) the association with the globulin fraction of the plasma; (*d*) the sensitiveness to the action of magnesium hydroxide and of certain azo dyes. The differences between the two substances are manifested by: (*a*) the relation to calcium (essential for the clotting process but not necessary for the action of complement); (*b*) the inactivation of prothrombin but not of complement by aluminum hydroxide and by heparin; (*c*) the finding of markedly anticomplementary properties in human serum without a disturbance of clotting.

I. DAVIDSOHN.

THE VENOMS OF NORTH AMERICAN PIT VIPERS. T. S. GITHENS, J. Immunol. **29**:165, 1935.

In twenty-six species, the amount of venom and the danger to man were directly proportionate to the size of the animal. Great differences were noted in the toxicity of venoms from closely related species. The nature of the toxic effect differed also; the venom of the primitive species attacked primarily nerve centers, while that of more highly developed species had a more pronounced effect on the blood and the blood vessels.

I. DAVIDSOHN.

TESTS FOR IMMUNITY TO EPIDEMIC POLIOMYELITIS. J. A. KOLMER, G. KLUGH JR. and A. M. RULE, J. Immunol. **29**:175, 191 and 199, 1935.

Antiviral Tests of Serum (J. A. Kolmer and A. M. Rule).—The technic for the demonstration of the neutralizing properties of blood serum is given. Effective resistance to poliomyelitis is indicated when 0.5 cc. of the serum neutralizes at least 10 minimal infective doses of monkey passage virus. On the other hand, the absence of such power is not synonymous with absence of resistance to poliomyelitis. The method outlined is the only one available for determining resistance to poliomyelitis. It requires the use of monkeys (Macacus rhesus), takes about three weeks and is therefore expensive.

Skin Reactions (J. A. Kolmer, G. Klugh Jr. and A. M. Rule).—Intracutaneous inoculation of suspensions of spinal cord from monkeys dying of poliomyelitis failed to provoke any characteristic skin reaction in man and monkeys treated with poliomyelitic vaccine and in normal control patients and monkeys. Mild local reactions are interpreted as due to the irritating effect of sodium ricinoleate.

Colloidal Gold Tests, Complement Fixation and Precipitation (J. A. Kolmer and A. M. Rule).—The serum colloidal gold test of Eberson proved valueless. Complement fixation with suspensions of virus-containing spinal cord from monkeys was negative with serum of man and of normal monkeys but was positive with that of a considerable number of monkeys inoculated with poliomyelitic vaccine or with mixtures of the vaccine and immune serum. Precipitation tests of serum with filtrates of spinal cord from monkeys dying of poliomyelitis were negative.

I. DAVIDSOHN.

THE PRODUCTION OF KOCH'S PHENOMENON WITH VARIOUS STRAINS OF TUBERCLE BACILLI. W. PAGEL, J. Path. & Bact. **41**:89, 1935.

In tuberculous animals intracutaneous injection of dissociated strains of tubercle bacilli (R and S forms) produced the Koch phenomenon in a definite and uniform manner. In animals with generalized tuberculosis R strains excited in the majority of cases an intense Koch phenomenon, while S strains either failed to produce the phenomenon or produced it only in an atypical form. Exceptions to this rule were met with. The S form of BCG and the human strain Ratti produced in a small number of animals already infected with avirulent tubercle bacilli and in some virulently infected animals before the occurrence of

generalization a small but definite Koch phenomenon. In the case of the R form of the human strain Ratti the ability to produce hypersensitiveness was connected with the virulence of this modification. Of the dissociated BCG strains both modifications were avirulent, but the R strain, which excited a more intense Koch phenomenon in allergic animals, also produced a more extensive local reaction in normal animals. Nonpathogenic acid-fast bacilli and killed tubercle bacilli were unable to produce the Koch phenomenon when they were used in the same dilutions as living and virulent tubercle bacilli. Rarely they excited hypersensitiveness in tuberculous animals when they were injected in excessive amounts.

FROM THE AUTHOR'S SUMMARY.

THE DECOMPOSITION OF GROUP-SPECIFIC SUBSTANCES BY BACTERIA. F. SCHIFF, Klin. Wchnschr. **14**:750, 1935.

The A substance in peptone and the group-specific substances A and B from human saliva were decomposed by many strains of the gas bacillus. A corresponding action by other bacteria, among which were strong peptolytic and proteolytic forms, could not be demonstrated.

BLOOD GROUPS AND DISEASE. F. SCHIFF, Klin. Wchnschr. **14**: 786, 1935.

Several authors have claimed that persons with blood of group B are less susceptible to poliomyelitis than those with blood of other groups because they found a smaller percentage of the former among those with the disease in comparison with the frequency with which blood of group B is found in normal persons. However, as Schiff points out, as a rule the difference is not statistically significant, and some authors have found a high frequency of group B in their poliomyelitic subjects. Other fallacies were that the populations studied were not homogeneous and that the so-called control group was not comparable with the infected group.

A. S. WIENER.

THE ANTIGENS AND ANTIBODIES OF TAPEWORMS. O. SIEVERS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:208, 1935.

Rabbits inoculated with mixtures of alcoholic extracts of Diphyllobothrium latum and of Taenia saginata with hog serum or with watery suspensions of tapeworm tissue produced immune serums that reacted specifically by means of the complement fixation test with alcoholic extracts of various tapeworms. Under proper quantitative conditions the immune serums reacted in a species-specific manner with the homologous antigens. The species specificity of the T. saginata antiserums was more pronounced in the precipitation reaction, while in the case of the D. latum the complement fixation reaction was more suitable. Attempts were made to demonstrate antibodies or antigens in the blood serums of persons suffering from infestation with D. latum. The results were not insufficiently specific to be of practical value.

I. DAVIDSOHN.

CUTANEOUS ALLERGY AND THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF INFESTATIONS WITH TREMATODES. O. WAGNER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:225, 1935.

The reactions to intracutaneous injections of extracts of the liver fluke (Fasciola hepatica) were not specific. They occurred in 73 per cent of infected sheep, but they also occurred in sheep free from the parasite. Positive reactions were observed also in sheep infested with other species of trematodes. The complement fixation test gave similar results, but a combined use of both procedures increased the incidence of positive results in the infected animals to 90 per cent. Both tests lack practical significance in view of the fact that examination of the feces makes it possible to establish the presence of the parasites quite easily. Wagner calls attention to a possible application of both tests in those cases of trematode infestation in man in which a homologous antigen is not easily obtainable.

I. DAVIDSOHN.

Tumors

GONADOTROPIC HORMONE IN TUMOR OF THE TESTIS. F. HINMAN and T. O. POWELL, J. Urol. **34**:55, 1935.

In tumor of the testis the fluid in the tunica vaginalis may contain more gonadotropic hormone than the urine. In most of the cases the tumor is embryonal in character. In such cases the urine contains gonadotropic hormone. The quantitative determination of the hormone before and after irradiation of the tumor may be helpful in control of treatment and in prognosis.

TERATOMA OF THE SPINAL CORD. P. C. BUCY and D. N. BUCHANAN, Surg., Gynec. & Obst. **60**:1137, 1935.

The tumor first caused symptoms at the age of 16 months but was not removed until the child was almost 3 years old and suffering from a mixed and alternating spastic and flaccid paraplegia. The tumor was cystic, contained 35 cc. of glairy material, showed a mural nodule and lay within the arachnoid. In common with the ten previously recorded examples of teratoma of the cord the growth contained ectodermal and mesodermal but no entodermal derivatives. The origin was traced to the posterior columns at the caudal end of the cord.

WARREN C. HUNTER.

DYSONTOGENETIC AND MIXED TUMORS OF THE UROGENITAL REGION. JOSEPH MCFARLAND, Surg., Gynec. & Obst. **61**:42, 1935.

From study of certain neoplasms in children and review of the literature McFarland concludes that the tumors developing in connection with the embryonal structures concerned in the formation of the urogenital sinus are best accounted for on the basis of dysontogenesis. The most characteristic of these tumors are "mixed tumors," since they contain several different tissues, some of which are foreign to the part. Even tumors of a more simple structure seem to belong in the dysontogenetic group by reason of their localization, clinical appearance and behavior. The frequent occurrence at a very early age and the distinctly embryonal nature suggest origin from residual embryonal remnants. All of the neoplasms are potentially malignant and most of them actively so. To consider these tumors as varieties of sarcoma is thought to be an error. The literature on the subject is confused by complicated terminology, incorrect identification of individual tumors, premature publication in cases in which the patients are supposedly cured, and the designation of uterine fibroids as malignant from structural appearances the significance of which is not understood.

FROM THE AUTHOR'S CONCLUSIONS. (WARREN C. HUNTER.)

PINEAL TERATOMA. A. J. MCLEAN, Surg., Gynec. & Obst. **61**:523, 1935.

McLean reviews the 25 recorded cases of pineal teratoma and reports a new case. The tumor was parapineal, measured 21 by 18 by 25 mm., and showed a remarkable array of tissues, including cysts lined with stratified epithelium, surrounded by sweat and sebaceous glands, also cysts lined by ciliated epithelium. Connective tissue, fat, striated muscle and ganglion cells were likewise present. The teratoma had a more complicated structure than any previously recorded example.

WARREN C. HUNTER.

THE GENERAL PATHOLOGIC CONCEPTION OF CANCER. JAMES EWING, Canad. M. A. J. **33**:125, 1935.

Ewing sums up the trend of cancer research as follows: During the past thirty-five years cancer research has focused steadily on the intrinsic properties of the cells and the forces that control them. The first impulse in this direction came

from the final establishment of the fact that the tumors of the lower animals are transplantable and that the cells survive. Substantial support of the exclusive importance of tissue cells came from the observation that the invisible rays of radium can destroy some tumors. Progress in genetics added impressive evidence that the essential phenomena of cancer may be referred to the potencies of cells and the factors resident in the host. Studies in the physiology of certain tumors had long revealed them as functioning organs, arising in response to functional demands, and not as vagrant cells or lawless unphysiologic overgrowths. In aerobic glycolysis and diminished respiration chemistry has revealed hitherto unsuspected and more or less distinguishing properties of cancer cells. Even the filtrable agents in fowl sarcomas seem likely to take their place as sporadic but significant expressions of cell potencies in this species. The behavior of embryogenic organizers and inducers, separable from the cells, opens up a new field in cell physiology which may be of much interest for cancer. Recent studies of the relation of hormones to tumors and the experimental demonstration that estrin, androtin and prolan produce in appropriate organs, excessive and even atypical overgrowths which readily run into cancer furnish convincing evidence that these processes when occurring spontaneously must also be assigned to the action of internal agents acting on the intrinsic properties of the cells. The chemical relationship between the cancerigenic coal tar products and hormones and vitamin D supports this view and makes it easier to accept other external chemical substances as the actual agents exciting cancer. The great variety of agents producing cancer, as with inflammation, indicates that their mode of action is not always the same, and the resulting process not always identical.

The missing knowledge in this field concerns the mode of action of the cancerigenic agents. Androtin and prolan A produce enormous overgrowth of the testes and comb of the cock but no tumors. Ovarian implants lead to marked overgrowth of mammary tissue in the mouse, but close analysis reveals that cancer does not develop until stagnation occurs. The deficiency of vitamin C in scurvy leads to remarkable changes in the skeletal muscles suggesting a sarcomatous tendency, but never to sarcoma. The exact mode of action of the highly cancerigenic coal tar products has not been traced but is probably indirect. All these agents act on the tissue cells to produce cancer, and their effects must be referred exclusively to intrinsic potencies of the cells, but how they induce the malignant change is unrevealed. The secret of malignant growth seems still to be enshrouded in the obscurities of intracellular life, where it will probably long remain.

FROM THE AUTHOR'S SUMMARY.

EFFECTS OF THE BETA RAYS OF RADIUM ON THE AGENT OF ROUS SARCOMA AND CERTAIN OTHER AGENTS. S. L. BAKER, Brit. J. Exper. Path. **16**:148, 1935.

A quantitative study has been made of the destructive effects of the beta rays of radium on the following bodies: Bacillus coli, Staphylococcus aureus, bacteriophage, filtrate of Rous sarcoma no. 1, tetanus toxin, spores of Bacillus anthracis, hemolytic amboceptor, guinea-pig complement, trypsin and lysozyme. The relative susceptibility of these various bodies to destruction by the beta rays has been estimated, and, in addition, figures for vaccinia, pepsin and invertin are deduced from other workers' results. The agent of the Rous sarcoma fell into the bacterial group, but tetanus toxin was equally susceptible. The antibodies were considerably more resistant to destruction and the ferments still more resistant. The evidence from these results as to the living nature of the agent of Rous sarcoma was equivocal but indicated that this agent is not of the nature of a ferment.

FROM THE AUTHOR'S SUMMARY.

THE NORMAL ESTROUS CYCLE AND THE RESPONSE TO ESTRIN IN TWO STRAINS OF MICE DIFFERING GREATLY IN INCIDENCE OF SPONTANEOUS MAMMARY CANCER. G. M. BONSER, J. Path. & Bact. **41**:33, 1935.

A line of albino mice in which a high percentage of the female members normally acquire spontaneous cancers of the breast was compared from the point of

view of the age of onset of estrus, the duration of the cycle, the duration of heat and the cessation of estrus with a line of agouti mice normally resistant to the development of cancer of the breast, and it was found that there was no demonstrable difference in the two strains. The two strains were also compared with regard to reactivity to estrin administered by various methods and found to be much alike. If anything, there was slightly greater reactivity on the part of the cancer-resistant strain.

FROM THE AUTHOR'S SUMMARY.

Medicolegal Pathology

DETERMINING THE GROUP OF HUMAN BLOOD-STAINS: NOTES ON AN ANOMALOUS GROUP O SERUM. D. HARLEY, Brit. J. Exper. Path. **17**:35, 1936.

The presence or absence of receptors A and B in human blood stains can be tested for by determining the ability or inability of the stain to absorb the corresponding iso-agglutinin from serum of group O. While performing such tests Harley encountered a rare serum of group O which exhibited the peculiar property that, while fresh blood or blood stains of group B removed only the anti-B iso-agglutinin, blood of group A removed both iso-agglutinins. He attributes this nonspecific absorption to some sort of coupling between the iso-agglutinins in group O serum. His observation of nonspecific absorption differs from those previously reported in that he observed nonspecific absorption occurring in one direction only. Of course, serums of this sort are not reliable for use in grouping blood stains for forensic purposes.

A. S. WIENER.

TRANSFUSION ACCIDENTS FROM THE MEDICOLEGAL STANDPOINT. P. MOUREAU, Ann. de méd. lég. **15**:951, 1935.

Moureau describes two reactions to transfused blood: 1. A woman of 35 with pernicious anemia which was responding well to liver therapy was given a transfusion. A physician typed the bloods of the patient (whose blood, he found, belonged to group O), her husband (group AB) and her father-in-law (group O). Despite the fact that these results contradicted Bernstein's theory, the father-in-law's blood was transfused, and the transfusion was followed by a typical hemolytic reaction. The bloods were reexamined immediately after the transfusion, and it was found that there had been an error in typing: The father-in-law's blood really belonged to group A.

2. A woman of 79 with a severe anemia of unknown cause was given a transfusion of 350 cc. of blood. Immediately afterward there was a chill, and the temperature rose to 101.6 F. Thereafter, up to the time of death, fifteen days after the transfusion, the temperature ranged from normal up to 102 F. Autopsy and the fact that the donor's blood was demonstrable in the patient's circulation proved that the transfusion probably had no connection with the patient's death.

Moureau points out that an accident which is due entirely to a preventable error in technic, as in the first case, may have medicolegal consequences in that the physician can be held liable for malpractice. In the second case, on the other hand, it was clearly demonstrated that the transfusion was not responsible for the reaction.

A. S. WIENER.

THE HISTOLOGY OF THE TESTICLES OF CASTRATED SEX OFFENDERS. KOOPMANN, Deutsche Ztschr. f. d. ges. gerichtl. Med. **26**:43, 1936.

In sixty-eight men castrated on account of various sexual offenses no characteristic histologic changes were found in the testicles.

Society Transactions

PHILADELPHIA PATHOLOGICAL SOCIETY

Jan. 9, 1936

MORTON MCCUTCHEON, *Presiding*

AMYLOIDOSIS OF THE THYROID AND OTHER VISCERA IN A PATIENT WITH CHRONIC BRONCHIECTASIS. O. NORRIS SMITH.

Of interest was the unusual distribution of the amyloid: Among other viscera, it was deposited in the thyroid, an organ rarely involved. The patient was a 36 year old white man who for ten years had been treated for supposed pulmonary tuberculosis. A few weeks before death he entered the Hospital of the University of Pennsylvania, where the pulmonary lesion was correctly recognized as extensive bronchiectasis. While in the hospital he died following an operation.

At autopsy there was widespread dilatation of the bronchial tree in the lower half of the left lung; this was associated with accumulation of purulent material within the dilated bronchi and much formation of scar tissue in the affected portion of the lung and pleura. The thyroid, which during life had gradually enlarged over a period of eight months, was found to be swollen to twice the normal size, tough in texture and of a pale pink homogeneous appearance. The adrenals were also about twice the usual size and grossly presented firm homogeneous cortical zones that suggested the presence of amyloid. The kidneys were enlarged and firm and had the appearance characteristic of amyloid disease; in addition, however, many surface scars indicated an associated arteriosclerotic process. The liver, spleen and pancreas disclosed no characteristic gross changes, though under the microscope amyloid deposits were found.

On histologic examination of the thyroid, the acini were found distorted and sparsely scattered in massive amyloid deposits interspersed with areas of adipose tissue. The adrenal showed replacement of nearly all the cortex by amyloid. In the kidneys patches of amyloid were observed in most of the glomeruli, in the walls of the vessels and along the basement membrane of many tubules. In the liver and pancreas only the small arteries were involved, while in the spleen a similar process was accompanied by a delicate and diffuse amyloid coating of the reticulum.

Review of the literature brings to light but few cases of massive deposition of amyloid in the thyroid. In two reported cases, at least, the causal disease was long-standing bronchiectasis. However, while massive amyloid deposits in the thyroid appear to be rare, amyloid involvement of its small vessels is not uncommon. There is nothing noteworthy about the distribution of amyloid in the other organs, except the sparse involvement of the liver.

CONGENITAL RACEMOSE BRONCHIAL ADENOMA OF THE RIGHT EPARTERIAL BRONCHUS WITH PULMONARY AGENESIA OF THE RIGHT UPPER LOBE. B. R. REITER.

A 61 year old Russian entered the Philadelphia General Hospital in February 1935. For fifteen years he had been troubled by constant sinus infection and six weeks before admission had an exacerbation of symptoms followed by a sudden onset of attacks of dyspnea. These occurred four or five times daily, each lasting from one-half to two hours. Simultaneously a persistent cough developed, which was productive of moderate amounts of green sputum; 20 pounds (9.1 Kg.) had been lost in two months. Twenty-two years previously the patient had for six months been confined to a sanatorium for patients with tuberculosis.

He was a fairly well developed and well nourished elderly man. The heart was apparently normal. The blood pressure was 144 systolic and 100 diastolic. The chest showed a deepened right infraclavicular fossa, and the anterior-posterior diameter of the chest was increased; wheezing expiratory râles were present throughout both lungs, and at the right apex there were dulness to percussion, increased intensity of breath sounds, amphoric in quality, and whispered pectoriloquy.

The blood and urine were normal. The Wassermann test was negative. Seven specimens of sputum were examined for tubercle bacilli, but none were found.

Roentgenologic Examination.—There was evidence of pansinusitis. There was slight deviation of the trachea to the right with irregular increase in markings and mottled radiolucent areas in the upper half of the right lung. Calcified nodes were visible in the upper mediastinum. The interpretation of these observations was: pulmonary tuberculosis with cavitation.

Postmortem Examination.—The heart showed moderate hypertrophy of the right ventricular myocardium. The lungs were markedly emphysematous, and there was diffuse bronchopneumonia in both lower lobes and in the markedly enlarged right middle lobe. The right upper lobe consisted of a small firm partially cartilaginous mass of tissue surmounted by a thin cap of normal pulmonary parenchyma. On sectioning, this mass was found to be made up of many small intertwining and communicating cystic cavities, some of which contained thin mucopurulent secretion. The largest was 1.3 cm. in diameter. The walls of these cavities were thick and consisted of firm fibrous and cartilaginous tissue. The right eparterial bronchus led directly into the anomalous bronchial labyrinth. Sections of the abnormal right upper lobe showed many small spaces lined by epithelium varying from cuboidal to columnar, the intervening septums being made up of fibrous tissue, which contained patches of cartilage and normally developed peribronchial mucous glands, large arteries and nerves. In some areas a few scattered patches of imperfectly developed alveoli were visible.

Cystic disease of the lungs is not uncommon. One may find the lungs studded by multiple thin-walled cysts of all sizes, usually much larger than in the present case. At times an entire lobe may be replaced by a single large cyst. In infants a somewhat similar condition may exist in which one lobe may be greatly enlarged by multiple thin-walled cysts, which are prone to rupture, giving rise to spontaneous pneumothorax. Regardless of the etiology of the condition in adults, one is forced to conclude that the infantile condition is due to some form of maldevelopment, but the type usually observed stands out in distinct contrast to the unusual type presented here in which all of the cysts are small thick-walled cavities, intercommunicating freely, with fully developed mucous glands, arteries and nerves, representing matted bronchial elements with agenesis of the pulmonary parenchyma.

It is believed that this condition is due to arrest of pulmonary development in the fetus at a fairly early stage. The explanation for the difference between this type and the more common type with multiple thin-walled cysts replacing normal parenchyma is not clear as yet, although this difference is probably due to arrest of development at different periods.

Clinical and Experimental Studies on the Pathologic Physiology of the Spleen, with Special Reference to the Indications for and the Results of Splenectomy. Charles A. Doan.

It was just thirteen years ago next month that I had the honor of presenting before this society the results of my first medical investigation on the relationship to hematopoiesis of the vascular, sinusoidal and capillary circulation in the bone marrow. To those interested in the problem of the physiology of the blood cells the parallel importance of cell distribution and of cell destruction to a knowledge of the genesis and delivery of these essential units is axiomatic. In 1923 Krumbhaar coined the term "hemolytopoietic equilibrium" to express this essential interrelationship. My investigations in the intervening years have included, therefore.

observations relative to certain extramedullary influences on the blood cells, and I wish to report some studies having to do with the hematopoietic equilibrium as influenced by splenic function.

My first investigations of the spleen were carried out at the Thorndike Laboratory, Boston City Hospital, in association with Zerfas, Warren and Ames, and had to do with its participation in the sequestration of granulocytes in experimentally induced leukopenic states in rabbits. With the spleen intact sodium nucleinate will precipitate a leukopenic state during which the granulocytes may be demonstrated as accumulating primarily in this organ (oncometer studies of splenic enlargement, differential counts of splenic cells, multiple serial biopsies). Following splenectomy the peripheral leukopenia is lessened in degree and duration or entirely eliminated. The classic studies of Barcroft on the function of the spleen as a reservoir for red blood cells were being made at approximately the same time. The importance of concomitant information on variations in the volume of the plasma when cell changes are studied was recognized in our first experiments, and with each cell count and hematocrit estimation the serum protein was determined. The red cells and hematocrit reading were observed to rise immediately after splenectomy, and the protein in the serum increased, indicating some loss in plasma volume.

More recently these earlier studies have been supplemented by direct determinations of blood volume on a different animal species, and the mechanism of postsplenectomy cellular and plasma readjustments has been subjected to rigid analysis. After eliminating the rabbit, cat and dog as unsuitable for our purposes, we found the sheep to have a large readily contractile spleen, similar in shape and relative in size to that of man, so that in animals weighing upward of 150 pounds (68 Kg.) it was possible to make serial determinations of blood volume by the vital brilliant red dye method, the coefficient of extinction of the dye being read in a spectrophotometer with appropriate controls permitting very accurate measurements. It has been possible to demonstrate a cell volume increase of from 226 to 742 cc. (23 to 63 per cent) of packed red blood cells following maximal epinephrine-induced contraction of the spleen, the original cell volumes ranging from 740 to 1,140 cc. The total number of red cells in the peripheral blood was increased by from 3,000,000 to 4,500,000 per cubic millimeter (the counts being 8,900,000 to 13,600,000), and the hematocrit reading by from 10 to 16 per cent. The plasma volume was decreased postoperatively in every instance by from 236 to 480 cc. The increase in the peripheral level of the red blood cells has been maintained indefinitely in those sheep in which it was followed up. Serial biopsy specimens from the sheep's spleen show before injection of epinephrine a tremendous number of erythrocytes in the parenchyma with widely separated follicles in sharp contrast to dense lymphoid follicles and an almost erythrocyte-free spleen after injection of epinephrine. Our animal studies have led us not only to appreciate even more fully than previously the capacity of the splenic parenchyma as a reservoir for blood elements but also to recognize in the normal mammalian spleen some inhibitory influence on bone marrow "poiesis."

Our first studies of human splenic function were based on well proved and long-established clinical facts. That good results were obtained from successful splenectomy in the patient with chronic congenital hemolytic jaundice has been recognized for many years, though the postsplenectomy increase in red cells has been described as occurring some weeks or months after operation, and the records of the mortality have been interpreted as a warning against operative intervention in acute hemoclastic crises. Our first clinical hematologic studies were made with a painstaking baseline investigation of selected patients for several weeks or months, and then, on the day of operation, studies of the blood were made at frequent intervals before and after ligation of the pedicle. An entirely unanticipated and spectacular change in the level of the erythrocytes was observed to occur on the operating table immediately after splenectomy. Later studies of the blood volume proved this to be not only the result of some decrease in the plasma volume but, more important, the reflection of a large and significant increase in the erythrocyte volume in the circulating blood. In fifteen consecutive patients with congenital hemolytic icterus this immediate rise of from 900,000 to 1,900,000 per cubic milli-

meter has occurred irrespective of the cell level preoperatively. Leukocytes and thrombocytes likewise increase after splenectomy, and this increase is maintained, changing absolute thrombopenia and the frequently encountered leukopenia to normal levels or above.

After the experiences cited we were prepared to consider from a somewhat different angle than before the patients with hemoclastic crises when they appeared. Following intensive liver therapy and blood transfusions without therapeutic benefit we have advised and now have seen successfully consummated six splenectomies in as many patients ranging in age from 4 to 56 years whose total red cell count at the time of operation was a million cells or under. All have survived, and all returned promptly to a normal hemolytopoietic balance. The erythroclastic crises in some instances occurred spontaneously; in others they were precipitated by infection or trauma. The cause of the erythroclastic crisis, the erythrocyte level or the obviously critical clinical condition of the patient was not sufficient to cause us to withhold operative intervention. The best possible medical preparation and postoperative care have been given, of course. In most of the cases my colleague, Dr. George Curtis, has performed the operation, but Drs. Verne Dodd, I. B. Harris, André Crotti and L. V. Zartman have operated successfully in individual cases in our series. The essentials of success are accurate diagnosis, adequate medical management and expert surgical technic.

The fact that removal of the spleen cures congenital hemolytic jaundice clinically can no longer be denied; the fact that in the absence of the spleen the bone marrow functions more efficiently in the production of granulocytes, erythrocytes and thrombocytes has been demonstrated in every patient; it has also been demonstrated that the size and fragility of the red cell return more nearly to normal. The spleen of the patient with congenital hemolytic icterus not only destroys erythrocytes but inhibits effective hematopoiesis.

Splenectomy has been performed in the treatment of other clinical entities as a part of our study of the pathologic physiology of this organ, and in every instance the hematologic studies have tended to confirm the general functional activities cited.

That the changes noted were not due to the anesthetic or to the operative trauma alone was proved by comparable studies on a series of fourteen patients undergoing major operations other than removal of the spleen: The cellular reactions both in kind and in degree were entirely dissimilar from those observed following splenectomy.

A fuller understanding of the pathologic physiology of the spleen will contribute materially to knowledge of the mechanism underlying the hemolytopoietic equilibrium and should form the basis for a more rational approach to therapy in many pathologic states whereby this system is disturbed.

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

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OSKAR KLOTZ, *President*

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RELATION OF THE DAILY LOSS OF IODINE IN URINE TO THE INCIDENCE OF GOITER. GEORGE M. CURTIS and NORMAN L. MATTHEWS (by invitation), Ohio State University.

The purpose of this investigation was to establish normal values for urinary iodine in central Ohio, which is in a region of moderately endemic goiter. Two hundred and forty determinations of the daily excretion of iodine in the urine of

ten persons revealing no evidence of thyroid disease were made. The twenty- four hour averages were from 36 to 78 micrograms. The grand average was 55 micrograms. The individual daily output of patients maintained on the usual hospital diet showed great variation; the output of persons taking a monotonous diet was, however, unusually constant.

Nine persons in New Orleans who were studied by the same methods (Moore) excreted from 60 to 270 micrograms of iodine daily and averaged 117. The average daily excretion of iodine by normal persons in central Ohio is likewise lower than the corresponding averages in the five goiter-free regions—Danzig, Vig-i-Sogn, Berlin, the Ligurian Coast and Effingen. It is higher, however, than the averages in three regions where goiter is prevalent—Norway, Hungary and Poland.

In central Ohio the lower daily loss of iodine in the urine thus corresponds to the higher incidence of goiter.

Demonstration of Thyrotropic Material in the Blood and Urine of Patients with Myxedema. Saul Hertz and Eric G. Oastler (by invitation), Massachusetts General Hospital, Boston.

A method of assaying thyrotropic hormone in the urine is described, and the results obtained by it in cases of thyrotoxicosis and myxedema are given. Pituitarectomized animals were used as the test objects.

Seasonal Variations in Human Spermatozoa. David L. Belding, Massachusetts Memorial Hospital, Boston.

An analysis of statistics on births in Massachusetts shows a seasonal fluctuation in the conception rate. This shows a rise in the period from June to October, inclusive, reaching a peak in September. This seasonal variation, though influenced by artificial factors such as the marriage rate, has been variously interpreted as the result of changes in the level of fertility in the male or the female.

The seminal fluid and spermatozoa of a healthy fertile male studied over a period of two years revealed no evidence of seasonal variation. Nonseasonal fluctuations in the volume of the seminal fluid and in the number and morphologic character of the spermatozoa were observed at times. The viability of the spermatozoa showed a seasonal fluctuation, being lowest during the summer months, the reverse of the conception rate. These negative results, though limited as to scope and standards, suggest that the seasonal variation in the conception rate is due to causes other than changes in the level of fertility in the male.

Effect of Iodides on Interstitial Mononuclear Pneumonia. Douglas H. Sprunt and Sara McDearman (by invitation), Duke University School of Medicine.

Iodine has been used in the treatment of granulomas, particularly syphilitic ones, with good results. In studying the so-called virus pneumonia (interstitial mononuclear pneumonia) one is impressed by the fact that although the lesion is not a focal accumulation of cells forming a granuloma it is composed of the same types of cells as a granuloma—monocytes and lymphocytes.

With this fact in mind it was thought worth while to study the effect of the iodides on mononuclear pneumonia. Thus far this work has been only partially successful, but it is thought that a preliminary report is warranted.

Adult rabbits free from Bacterium bronchisepticum and from Bacterium lepisepticum were used. The Levaditi strain of vaccine virus was employed. This virus was obtained from Dr. T. M. Rivers. Staphylococcus aureus was also employed since the toxin had been shown to produce a mononuclear pneumonia. The virus and the toxin were injected intratracheally. Isotonic sodium iodide was injected subcutaneously. The animals were killed between three and five days later.

Experiment 1.—Five rabbits were given daily injections of the iodide for one week before the toxin was administered and until the day they were killed. The resultant lesions differed neither grossly nor microscopically from the lesions produced in three controls.

Experiment 2.—Twelve rabbits were given various doses of iodide, starting at the time the virus was given. This treatment also failed to produce a modification of the lesion.

Experiment 3.—Since any animal's tolerance of iodide rapidly increases, it was decided to increase the dose by one-third each time. As it was found that the rabbits could withstand 100 mg. per kilogram, it was decided to start with this dose. The first dose of the iodide was given in some instances just before the virus or the toxin was given and in others twenty-four hours later. As there were no significant differences in the results between these two methods the results are discussed together. Nine doses of the iodide were administered, three being given each day.

Thirty-two rabbits were given the toxin of Staph. aureus intratracheally in sufficient strength to produce mononuclear pneumonia. Twelve of these rabbits were kept as controls, and twenty were given sodium iodide. The results are best shown in the accompanying table.

Effect of Treatment with Iodide on Production of Pneumonia

		Extent of Pneumonia					
		0 or Plus		2 Plus		3 Plus	
Rabbits	Type of Pneumonia	Rabbits	Per Cent	Rabbits	Per Cent	Rabbits	Per Cent
12	Staph. aureus toxin (untreated)...	0	0	3	25	9	75
20	Staph. aureus toxin (treated with iodide).....	13	65	4	20	3	15
9	Vaccinia virus (untreated).....	0	0	2	22	7	68
12	Vaccinia virus (treated with iodide)	7	58	4	33	1	8

A white blood cell count and a differential count were made daily on each rabbit. The percentage of polymorphonuclear cells was found to be elevated as a rule in the treated animals and depressed in the controls, but owing to the great variation in the counts it is difficult to know what significance to attach to this observation.

Further experiments are being carried on, in which the rabbits are being kept alive longer and extensive studies made of changes in the bone marrow.

Conclusion.—Sodium iodide decreases the extent of the pneumonic involvement in rabbits which have been given intratracheally the toxin of Staph. aureus or the virus of vaccinia.

PRODUCTION OF PULMONARY ABSCESS IN THE MONKEY BY THE BRONCHIAL ROUTE. CHARLES WEISS, Mount Zion Hospital, San Francisco, and University of California.

Employing a technic which prevents expulsion of the inoculum during coughing, I have produced pulmonary abscesses in Java monkeys (Macacus irus). The material inoculated was obtained at operation directly from the lung of a patient with chronic pulmonary abscess, and the anaerobes (Bacterium melaninogenicum, spirochetes, various types of fusiform organisms and anaerobic streptococci) were kept alive by frequent passage through the inguinal tissues of guinea-pigs. With the aid of a fluoroscope and a radiopaque ureteral catheter, pus from the lesions of the guinea-pigs was introduced into the bronchus of a lower lobe of the lung and the bronchus then blocked with a plug of agar.

Of fourteen monkeys thus prepared, six presented each a large putrid abscess, and one, pneumonia with minute multiple abscesses. These lesions were studied

during life by frequent roentgenography and histologically at necropsy. Several control inoculations of heat-sterilized pus and agar resulted in transient atelectasis without evidence of suppuration. The lesions obtained by injecting pure cultures of the aforementioned anaerobes are noted and described.

The data suggest that pulmonary abscess may, in certain cases, result from aspiration of infectious material (probably derived from the upper respiratory passages) with plugging of the corresponding bronchus.

THE HEMOCYTIC CONSTITUTION OF THE RABBIT AS AN INDEX OF ITS NATURAL RESISTANCE TO MALIGNANT TUMORS. ALBERT E. CASEY, LOUISE PEARCE and PAUL D. ROSAHN, Rockefeller Institute for Medical Research, New York, and University of Virginia.

Hereditary and seasonal variations in the blood formulas of healthy adult male rabbits have been reported from this laboratory, as well as hereditary and seasonal variations in the natural resistance of healthy adult male rabbits to the Brown-Pearce tumor. The present paper reports a series of sixteen experiments extending over a period of five years and designed to determine whether the variation in the individual blood formula is related to the variation in the reaction of the rabbit to this tumor.

Approximately four red blood cell counts, five white blood cell counts, four determinations of hemoglobin and a differential count of 1,000 white blood cells were made on each of 181 healthy young adult male rabbits prior to inoculation of the Brown-Pearce tumor. The counts were made over an average period of from one to two weeks. On 121 of the rabbits blood platelet counts were also made, averaging four on each rabbit. From the various determinations on each animal its mean blood formula was constructed. Then 0.3 cc. of an emulsion of the tumor was inoculated intratesticularly in each animal.

Five animals died of intercurrent disease and were eliminated from the analysis. Animals that died of the tumor were said to have a mortality of 100 per cent. Surviving animals were killed at the end of sixty days and were classified as follows: those having neither a primary nor a metastatic tumor at postmortem examination—a mortality of 0 per cent; those having a primary tumor but no metastases at postmortem examination—a mortality of 33 per cent, and those surviving the sixty day period but having metastases at postmortem examination—a mortality of 67 per cent.

A statistically significant relationship was found between the preinoculation levels of the various blood cells and of hemoglobin and the subsequent reaction of the animal to the tumor. Low levels of basophils, neutrophils, monocytes, white cells as a whole and platelets were associated with resistance to the tumor; high levels were associated with susceptibility. Low levels of hemoglobin, red cells, lymphocytes and eosinophils were associated with susceptibility and high levels with resistance. In the case of the red cells, platelets and lymphocytes there was an additional striking relationship: At the modal point for these three factors the animals were distinctly resistant. Resistance declined proportionately for a distance of one and one-half times the standard deviation either above or below the modal point. Coefficients of correlation with the mortality of the class frequency in this range varied from 0.7 to 0.9 and were significant.

In previous studies little relationship was found to exist between the mean levels of the various blood cells, the mean level of the hemoglobin and the mean level of the blood platelets. This was reflected in the present studies by a remarkable lack of uniformity as regards the mortality indicated by the various blood factors in a given animal. In no animal did all the factors indicate susceptibility or resistance. When nine or more of the fifteen blood factors indicated susceptibility the mortality averaged 87 per cent; when only one or two of the fifteen blood factors indicated susceptibility the resulting mortality was 14 per cent. A linear regression line extended between the two extremes and could be projected to 0 per cent mortality for 0 points of susceptibility.

TISSUE IMMUNITY: THE TISSUES IN ACTIVE AND IN PASSIVE IMMUNITY. REUBEN L. KAHN, University of Michigan.

It is well known that the capacity for antibody reactions and for skin reactions shown by protein-immunized animals can be passively transferred to nonimmunized animals. This fact has led to the belief that the passively immunized animals possess potentially the same degree of immunity as the actively immunized ones. By means of the antitoxin-toxin method which I have reported it was found that the tissues of actively immunized animals show marked differences in their capacity to react with antigen—the skin, for example, manifesting a capability from ten to fifteen times that of skeletal muscle—but that the tissues of passively immunized animals show relatively small differences in their limited capability of reacting with antigen. Furthermore, precipitin-free serum obtained from an animal during the period of incubation exerts practically no immunizing effect on the tissues when injected into a normal animal, although the tissues of the actively immunized animal from which it was obtained showed definite capability of reacting with the antigen. These observations suggest that the tissues of an actively immunized animal possess at least two types of immunologic capabilities; one type is transferable by means of antibodies, and the other type is an inherent part of the tissues and is not transferable.

PERMEABILITY OF THE CAPILLARIES IN INFLAMMATION. VALY MENKIN, Harvard University.

Earlier studies demonstrated that foreign substances, including a vital dye, graphite particles, a foreign protein and bacteria, introduced into the circulation, rapidly accumulate in an area of acute inflammation. This was shown to be in large part the result of an injury to the endothelium inducing an increase in the permeability of the capillary wall. This reaction of the capillary occurs in the earliest phase of inflammation, for the accumulation of trypan blue from the circulation has been demonstrated as early as two minutes after the injection of certain irritants into normal cutaneous tissues.

The present study indicates that the inflammatory exudate obtained by injecting turpentine into dogs contains a substance which when introduced into the normal skin of rabbits causes a prompt increase in the permeability of the capillaries as shown by the accumulation of trypan blue from the circulation. The effective substance differs in its properties and behavior from histamine in two respects: (1) Whereas injection of a cell-free exudate into an area of the skin is followed immediately by an intense and homogeneous accumulation of the dye from the blood stream in that area, this same exudate, unlike histamine, fails to produce contraction of the guinea-pig uterus or intestine or of the rabbit intestine. (2) When the exudate is injected into an area of the skin the dye diffuses uniformly in that area. When, on the other hand, 1 mg. or more of histamine is injected, trypan blue accumulates only at the periphery of the treated area of skin, while injection of 0.5 mg. or less of histamine results only in blanching of the central part of the treated area of skin, and the dye either fails to enter or stains only the periphery.

The active substance precipitated by treating the exudate with saturated ammonium sulfate or with 20 per cent di-sodium sulfate dissolves readily in water, saline solution or phosphate buffer. The possible effect of sulfate ion ($SO_4=$) has been ruled out. The cell-free exudate loses a considerable amount of its potency after dialysis, for the dye either fails to accumulate or does so only at the periphery of the area of skin treated with the dialyzed exudate. Dialysis of the fraction precipitated with ammonium sulfate causes complete loss of potency on the part of the protein material within the cellophane bag. The aggregated crystalline material obtained from the evaporated dialysate, redissolved in small amounts of distilled water, induces moderate accumulation of dye in the treated area. This indicates that the active material is probably crystalloid and tends to dialyze outward. The potency of the exudate is apparently not affected by variations in the hydrogen ion concentration ranging in p_H from about 6.5 to 7.4. Heating to 60 C. for two hours weakens the potency of the exudate, but exposure

to —20 C. has no effect. The exudate remains active in dilutions of about 1:10. Preliminary studies indicate that dilute concentrations of potassium or sodium cations fail to elicit the reaction, but that possibly the active substance is a derivative of ammonia. When crystalline serum albumin, which in itself has no potency, or the ineffective dialyzed ammonium sulfate precipitate of the exudate is treated with urease or trypsin and then injected, accumulation of the dye follows. These experiments suggest that the active substance causing increased permeability of the capillaries in inflamed areas of the skin of rabbits is not histamine but may be an intermediary product of protein catabolism. These studies are being continued, partly in an effort to purify and identify the crystalline material obtained from the dialysate of inflammatory exudate.

When the exudate is ashed, it is found that the nonvolatile inorganic material is active, inducing an immediate increase in the permeability of the capillary wall. Chemical and physiologic studies indicate that these inorganic substances are potassium and magnesium. The tentative conclusion is drawn that there are several active substances involved: (1) inorganic substances, namely, potassium and magnesium, and (2) possibly organic ones, crystalloid in nature.

CHANGES IN THE CUTANEOUS LYMPHATIC VESSELS AND THE LYMPH FLOW UNDER NORMAL AND UNDER PATHOLOGIC CONDITIONS. PHILIP D. MCMASTER, Rockefeller Institute for Medical Research, New York.

Vital dyes injected intradermally enter the lymphatic capillaries directly and render them visible. The dyes appear as well in the draining lymphatic vessels later on. This fact offers a method by which one may perceive the state of the lymphatic vessels and the rate of flow within them.

In a dependent limb or one subjected to partial obstruction of the veins the flow of lymph stops, although fluid in the limb increases. Excessive lymph flow appears during the reactive hyperemia that follows release of a complete obstruction of the blood vessels, whether or not the limb has previously been engorged with blood. In the ischemic patches which appear in the skin of a limb during total circulatory obstruction (Bier's spots) the lymphatic capillaries are somewhat contracted, whereas they are slightly dilated in the purple, congested skin. On release of the obstruction there occurs a strikingly rapid drainage of lymph from both regions.

The lymph flow in the edematous skin of nephritic patients is extraordinarily rapid. This flow is especially pronounced during periods of diuresis but is much greater than normal even during the period when the edema forms. The lymphatic capillaries in regions of cardiac edema undergo a notable dilatation, and the lymph within them stagnates. The valves of the lymphatic vessels may become incompetent, as shown by the retrograde flow on injection of a dye.

THE DURATION OF ASPHYXIA TOLERATED BY VARIOUS ORGANS: LOCAL AND GENERAL EFFECTS. FREDERICK M. ALLEN, Metabolic Institute, Morristown, N. J.

Temporary circulatory occlusion (clamping of blood vessels) is tolerated for periods of from a few minutes to four hours by different internal organs and for as long as twelve or thirteen hours by the limbs. This knowledge has proved useful in producing graded and controllable degrees of acute inflammation and subsequent sclerosis. The systemic effects of the ligation of a large mass such as a limb seem to offer a convenient and controllable form of shock of various degrees.

INTERNAL SECRETIONS AS A CAUSE OF CANCER. LEO LOEB, E. L. BURNS (by invitation), V. SUNTZEFF (by invitation) and MARIAN MOSKOP (by invitation), Washington University, St. Louis.

Two factors in mammary carcinoma in mice have been established: (1) the action of ovarian hormones and (2) a hereditary factor determining the responsive-

ness of the mammary gland to stimulation by these hormones, primarily by estrin. The significance of the action of internal secretions can be demonstrated by diminishing the amount of hormones acting as well as by increasing the amount. These methods give concordant results: The greater the amount of hormones acting continuously the greater is the number of animals in a certain strain which become cancerous. By eliminating the action of ovarian hormones altogether, the cancer rate becomes zero. The appearance of cancer is preceded by a preparatory period, during which ordinary metabolic or functional stimuli are able to induce cancer, provided previous to the preparatory period a sufficient quantity of ovarian hormones had a chance to act on the mammary gland. In the majority of cases the development of cancer is preceded by the production of a large cell acinar tissue, which either shows secretion or propagates in the form of tubules if it is further stimulated by hormones. The stronger the hormone action on this tissue the greater is the number of acini which become converted into cancer; likewise, the greater the hereditary responsiveness of the tissue to stimulation the greater is the number of developing cancers. Small doses of hormones, even if given over long periods of time, are as a rule ineffective. The responsiveness of mice to small doses of hormones does not essentially differ whether the mice are from a strain showing a high rate or from a strain showing a low rate of occurrence of tumors. However, the difference in the responsiveness of these strains to stimulation by hormones becomes manifest if one compares the readiness with which the large cell acinar tissue develops in strains with differing rates of tumor incidence.

Also in the vagina and uterus abnormal proliferation of the epithelial tissue can be produced through long-continued injection of estrin, and in the upper portion of the vagina conditions may develop not unlike those which in the human organ would be considered as early squamous carcinoma. In two cases, spindle cell sarcoma appeared in skin pierced by the needle at the time of the injections.

Hormones like carcinogenic hydrocarbons and other carcinogenic agents produce cancer by the often repeated or long-continued induction of processes of growth which ends in a change of the tissue to a new equilibrium. However, hormones differ from certain other agents in that as a rule they affect only those tissues on which they act under normal conditions, while the majority of other factors may affect indiscriminately many tissues with which they come into contact. Because hormones are substances to which the organism is adapted they may call forth pure stimulation as a step toward cancerous transformation, in contrast with agents such as tar, carcinogenic hydrocarbons and roentgen rays, which may alter also the stroma underlying the affected epithelium. Thus when other agents act complications arise which are lacking when hormones act, and these complications may obscure the mode in which the other agents transform normal into cancerous tissues.

CARCINOGENESIS: III. ISOMERS OF CHOLANTHRENE AND METHYLCHOLANTHRENE. M. J. SHEAR, Office of Cancer Investigations, United States Public Health Service, Harvard University.

In the investigation of carcinogenic compounds which is being carried on jointly with Prof. L. F. Fieser and his colleagues of the division of chemistry of Harvard University, further experiments have disclosed that 8, 9-di-methylene-1, 2-benzanthracene is less active than cholanthrene, of which it is an isomer. On treatment of mice with cholanthrene, tumors appeared in one half of the animals during the third month and in the other half during the fourth month. The isomer produced, during the first four months, only one tumor in fourteen mice; another tumor appeared during the fifth and four more during the sixth month.

Likewise, 7-methyl-8, 9-di-methylene-1, 2-benzanthracene is less active than methylcholanthrene, of which it is an isomer. With the use of methylcholanthrene, tumors appeared in almost all of the mice during the third and fourth months, whereas with the isomer no tumors were obtained in eleven months.

While methylcholanthrene has about the same activity as cholanthrene, addition of another methyl group to form 16, 20-di-methylcholanthrene results in a reduction of activity, as shown by a definite increase in the latent period.

Similarly, while 4-methyl-1, 2-benzpyrene is strongly carcinogenic, it has a longer latent period than benzpyrene. These results are analogous to those obtained by the English workers on introducing methyl groups into the molecules of active compounds.

Likewise 1, 9-methylene-1, 2, 5, 6-di-benzanthracene, which is related structurally to both methylcholanthrene and 1, 2, 5, 6-di-benzanthracene, is carcinogenic, but the latent period is at least as long as that of di-benzanthracene.

THE LATENT PERIOD IN EXPERIMENTAL CARCINOGENESIS. S. BURT WOLBACH, Harvard University.

An attempt was made to ascertain what processes were initiated by two carcinogenic agents antecedent to the development of tumors. Di-benzanthracene was introduced into the subcutaneous tissues of mice in pellets of cholesterol after the method of Shear, and 5-amino-2-azotoluene was employed in feeding experiments on rats after Yoshida. Both agents are destructive. With di-benzanthracene, the tissues surrounding the material were kept in a constant state of repair. Encapsulation with fibrous tissue did not occur as with chemically related controls. The 5-amino-2-azotoluene is toxic for the liver, and the effect of its long-continued administration was to produce degenerative lesions which were repaired by regeneration of the hepatic cells.

The indications are that the continuous reparative proliferation is the important factor and that the chemical composition of the agent is not specifically concerned in the production of the tumor other than in creating and maintaining a nonhealing process.

PROTECTIVE ACTION OF SULFHYDRYL AGAINST CARCINOGENESIS BY 1, 2, 5, 6-DI-BENZANTHRACENE. STANLEY P. REIMANN and EDITH M. HALL (by invitation), Lankenau Hospital, Philadelphia.

This article will be published in full in a later issue of the ARCHIVES.

NEOPLASTIC HYPERPLASIA OF THE GASTRIC MUCOSA IN RATS: PRODUCTION WITH LOW CASEIN DIET; PREVENTION WITH CYSTINE. GEORGE R. SHARPLESS (introduced by F. W. HARTMAN), Henry Ford Hospital, Detroit.

Young rats fed on a purified diet containing 4 per cent casein as a source of protein invariably show within three months' time hyperplasia of the lining epithelium of the rumen. This hyperplasia ranges from slight thickening and papillomatous formation to marked keratinization with the formation of epithelial cysts and marked activity of the epithelium, which breaks through the basement membrane. In rats fed diets containing 8 per cent casein hyperplasia is also produced but the changes are not so striking and require four or more months for development. Twelve per cent casein in the diet is sufficient to maintain the epithelium in a normal state.

The type of carbohydrate used in the diet (starch, dextrin or sugar) has little effect on the development of the lesion.

Deficiency of vitamin A, deficiency of vitamin B and a low intake of food are not apparent factors in causing the lesion.

Hair, which may act as an irritant, was constantly found in the stomachs of the normal and control animals on all the diets.

The addition of cystine to the 4 per cent casein diet prevents completely the hyperplasia described.

THE PHOSPHOLIPID METABOLISM OF TUMORS. FRANCES L. HAVEN (introduced by W. R. BLOOR), University of Rochester, N. Y.

The phospholipids of the normal cell have been assigned various functions, namely, those of fat metabolism, of oxygen transport and of cellular structure. Recently, Sinclair has shown that at least two types of phospholipid may exist, one functioning as an intermediate in fat metabolism and the other functioning in cellular structure. The liver has been shown by him to contain both types, while muscle contains mainly nonmetabolic phospholipid. This conclusion was reached by feeding elaidin and showing that elaidic acid replaces part of the normal phospholipid fatty acids of the rat and that its entrance into and disappearance from the phospholipids are rapid in the liver but comparatively slow in the muscle.

The phospholipid content of tumors is high, but the exact function of phospholipids here, as in normal cells, is unknown. The functions mentioned for normal cells possibly explain the permeability, the respiration or the low respiratory quotients of tumors.

By including elaidin in the diet of rats inoculated with carcinosarcoma 256, it was found that elaidic acid replaces some of the normal fatty acids in tumor phospholipids. Moreover, the entrance of elaidic acid into and disappearance from tumor phospholipids are comparatively slow. Therefore, the phospholipids of tumors, unlike those of the liver, have little if any metabolic function. Instead, they resemble those of skeletal muscle in being mainly of the nonmetabolic type.

THE VITAMIN C CONTENT OF TUMOR TISSUE. R. R. MUSULIN (by invitation), GLADYS E. WOODWARD (by invitation), ETHYL SILVERBLATT (by invitation) and C. G. KING, University of Pittsburgh.

Three week old tumors of rats, Philadelphia sarcoma 1, relatively free from necrotic areas and rich in cevitamic acid, were kept in carbon dioxide snow for periods of from two to five days without significant loss in indophenol titration values. Finely crushed samples were titrated chemically by the method of Bessey and King (modified by adding 2 per cent metaphosphoric acid), and a standard suspension was fed to guinea-pigs in quantities corresponding to 0.25 and 0.50 mg. of vitamin C per day. The response in growth and the degree of protection from scurvy agreed with the titration value within the limits of biologic assay, in comparison with animals receiving a standard solution of the pure vitamin. The indophenol titrations were further checked by destruction of the cevitamic acid by means of the specific oxidase described by Tanber and Kleiner: The dye titration, but not the iodine titration, approached zero at a rate corresponding with the oxidation of vitamin C.

FURTHER STUDIES ON CARCINOMA OF THE KIDNEY IN THE FROG. BALDUIN LUCKÉ, University of Pennsylvania.

Adenocarcinoma of the kidney is of common occurrence in leopard frogs (*Am. J. Cancer* **20**:352, 1934; **22**:326, 1934). In a large proportion of the tumors, many of the cells have acidophilic intranuclear inclusions, a fact which may be regarded as presumptive evidence of the activity of a virus. Otherwise these tumors closely resemble carcinoma in man both in structure and in invasive tendency; metastasis, though less frequent than with most forms of carcinoma in man, has been observed in a considerable number of cases.

The present report deals with the results of the first series of experiments in transmission and with the behavior of the adenocarcinoma in cultures. Over 1,400 frogs have been examined; approximately 700 of them had received injections of material from a number of different tumors by various routes (by way of the muscles, through the cranial cavity, by way of the lymph sacs, and through the pleuroperitoneal cavity); the others had been kept under identical conditions and served as controls. Growths at the site of injection were rare and soon retrogressed. However, tumors frequently developed in the kidneys. These renal tumors

were morphologically identical with the spontaneous carcinomas; their occurrence did not depend on any particular site of injection; their frequency increased with the length of survival of the animals. Thus, in frogs that died within three months after the injections the incidence of renal tumors was approximately the same as in the control group, that is, less than 2 per cent. In the group that had lived over one year the incidence reached 25 per cent.

These results are consistent with the hypothesis that with the injection of the tumor material an agent is transmitted which is organ-specific and probably of the nature of a virus.

Cultures made in a medium of frog plasma, chicken plasma and chick embryo extract grew rapidly, the outgrowths forming broad membranous sheets. The cells and their nuclei varied considerably in size; mitotic figures were fairly common. This is believed to be the first tumor from a cold-blooded animal that has been grown in cultures.

EFFECT OF SCREENED RADON ON MITOSIS. SHIELDS WARREN, New England Deaconess Hospital, Boston.

The mitoses of Walker rat carcinoma 256 were studied following gamma irradiation by means of a cervical applicator of the standard type, containing radon in a dose adequate for the treatment of tumors in man. Marked variation in the response of the dividing cells to irradiation was observed, even in the same strain of transplantable tumor carried in an inbred stock of homogeneous genetic constitution. This wide variation may explain some of the discrepancies in the results obtained in beta and in roentgen irradiation as well as some of the discrepancies obtained in clinical treatment. The drop in frequency of mitosis is rapid, occurring within one and one-half hours after the application of radon and reaching its lowest point during a period of from two to ten hours after irradiation. There is then a partial recovery to about a third of the number of mitoses previously present, a level which continues up to seventy-two hours. From two hours on, numerous abnormal mitotic figures are encountered.

THE PATHOGENESIS OF LYMPHATIC LEUKEMIA IN THE MOUSE. JOSEPH VICTOR, JAMES S. POTTER (by invitation) and MARGARET R. PREST (by invitation), Columbia University.

Morphologically normal lymph nodes of only one of five inbred strains of mice studied had increased rates of glycolysis at from six to eight months as compared with the rates at from six to eight weeks. This exceptional strain was C 58, which shows 90 per cent with spontaneous incidence of, and 100 per cent with susceptibility to, certain transmission lines of lymphatic leukemia. The morphologically normal tissue with a trend toward leukemic metabolism suggested potential malignancy of these tissues. This was tested as follows:

Lymph nodes were removed under ether anesthesia from twenty-four mice of strain C 58 at monthly intervals from the age of 2 to that of 12 months. Their Q_{O_2}, $Q_{CO_2}^{O_2}$ and $Q_{CO_2}^{N_2}$ [these symbols mean, respectively, the cubic millimeters of oxygen consumed per milligram of dry weight per hour, and the cubic millimeters of carbon dioxide equivalent to acid production per milligram of dry weight per hour aerobically and anaerobically. J. V.] were measured; then half of each node was injected into a mouse of strain C 58 (aged from 6 to 8 weeks); the remainder were fixed for histologic examination. There were 768 metabolic measurements.

The metabolism of all the lymph nodes decreased up to the age of 6 months. At various intervals after this in different mice the aerobic and anaerobic glycolysis of the nodes increased. This increase occurred in leukemic tissue and in morphologically normal tissue that transmitted the disease. Occasionally the increased glycolysis was not associated with transmissibility. With the development or advancement of leukemia transmissibility appeared. In no case did transmissibility occur without an increased rate of glycolysis.

One mouse, normal at autopsy, had a morphologically normal node that transmitted leukemia at the age of 11 months (high glycolysis) but not at that of 12 months (lower glycolysis).

The metabolic change of morphologically normal lymphoid tissue in the direction of malignancy is an expression of malignancy.

CHANGES IN THE BLOOD DURING PREGNANCY. FRANK H. BETHELL (introduced by RAPHAEL ISAACS), Simpson Memorial Institute, University of Michigan.

The blood of fifty pregnant women was examined at intervals throughout the latter months of pregnancy. The subjects, institutionalized, were under constant supervision and received regulated diets.

Simple anemia occurred frequently in this group, and its development followed a definite course: A moderate decrease in the number of erythrocytes and in the hemoglobin, with an approximately normal mean corpuscular volume, occurred in the second trimester of pregnancy which may be accounted for by hydremia. A further reduction in the number of the red blood cells and in the hemoglobin to an extent which cannot be accounted for solely by dilution of the blood indicates incomplete compensation for the physiologic destruction of blood. Such an anemia may be designated as hypoplastic or aregenerative. Subsequent stimulation of erythropoiesis, whether spontaneous, due to anoxemia or induced by therapeutic measures, results in a regenerative phase of the anemia, characterized by hypochromia, microcytosis and an increase in the number of reticulocytes. Unless treated, such anemia becomes increasingly severe throughout the latter months of pregnancy and persists after delivery.

COMPARATIVE STUDIES ON TRAUMATIC SHOCK PRODUCED EXPERIMENTALLY UNDER ETHER AND UNDER SODIUM AMYTAL ANESTHESIA. S. F. SEELEY (by invitation), H. E. ESSEX (by invitation) and F. C. MANN, Mayo Clinic.

The time of the onset of traumatic shock was determined following manipulation of the intestines for thirty minutes in dogs under ether alone, under sodium amytal alone and under combinations of these anesthetics. When the blood pressure had declined to 70 mm. of mercury the animals were considered to be in a state of shock. It was found that sodium amytal when administered alone or when administered before or after ether definitely delayed the onset of shock and death as compared with ether administered alone. The loss of fluid from the circulation in the form of saliva and in the form of exudate from the surface of the traumatized intestine was less rapid when sodium amytal was used alone or in combination with ether.

EXPERIMENTAL PULMONARY EDEMA. VIRGIL H. MOON and DAVID R. MORGAN (by invitation), Jefferson Medical College of Philadelphia.

No simple method for producing pulmonary edema has been described hitherto. The regular observation of this condition at postmortem examination following shock in man and experimental shock in dogs suggested means for its experimental production. Marked diffuse bilateral pulmonary edema was obtained by sublethal intoxication with various chemical agents, including histamine, sodium glycocholate, barbital, etc., by the introduction of bile or of muscle substance into the peritoneal cavity, by intestinal obstruction and with burns of the skin. The edema had the same characteristics as seen regularly in patients who suffer from similar intoxications. A hemoconcentration of from 25 to 40 per cent was present regularly. The edema fluid had a specific gravity equal to that of the blood plasma.

Pulmonary edema of this type results from increased permeability of the capillary walls and may be produced by various agents or conditions which produce injury to capillary walls. It regularly accompanies the syndrome of shock and frequently initiates terminal pneumonia in experimental animals and in man.

THE ADRENAL MEDULLA AND CORTEX IN RELATION TO EFFECTS OF INSULIN. B. N. BERG, J. GROSS (by invitation), C. LOWENBERG (by invitation) and T. F. ZUCKER (by invitation), Columbia University.

Dogs and cats in which the medullas of the adrenal glands have been completely removed by excision are convulsed by insulin in doses of from 0.5 to 2 units per kilogram injected intravenously. These doses are nonconvulsive for normal animals. The effects of smaller doses (0.1 and 0.25 units) in medullectomized animals do not differ from those observed in normal animals. In medullectomized animals all doses give the usual drop and recovery in blood sugar even in the presence of convulsions. Convulsions, therefore, are not synonymous with an inability of the blood sugar to return to the initial level. The greater tendency to convulsions may be due to the removal of the medulla or to an injury of the cortex.

Immediately after bilateral adrenalectomy, the course of the action of insulin does not differ from that observed in the normal animal. Seventy-two hours later, insulin produces convulsions, and the animal dies in hypoglycemia. In recovery experiments on completely adrenalectomized animals kept alive with cortical extract, convulsions occur with 0.1 unit per kilogram. The effects on the blood sugar show all gradations from hypoglycemic death to normal recovery, apparently according to the adequacy of the treatment with cortical extract. The observations indicate that the adrenal gland per se as part of an emergency reflex mechanism is not required for the recovery of blood sugar, but a factor derived from the cortex is necessary. In the absence of naturally stored substance or of injected cortical extract a return of the blood sugar does not occur. The dosage of cortical extract in these experiments was approximately from 2 to 5 dog units per kilogram per day—from two to five times the requirement for maintenance. Whether the factor dealt with is identical with the life-preserving cortical factor and whether it plays a rôle in the ordinary events of carbohydrate metabolism (i. e., when there is no injected insulin) remain to be seen.

A TOXEMIA OF PREGNANT RABBITS WHICH IS ASSOCIATED WITH TRANSMISSIBLE HEREDITARY ABNORMALITIES PROBABLY OF ENDOCRINE ORIGIN. HARRY S. N. GREENE (introduced by WADE H. BROWN), Rockefeller Institute for Medical Research, Princeton, N. J.

A certain disorder in pregnant rabbits presents a characteristic clinical and pathologic picture and in many respects resembles the toxemia of pregnancy in woman. The most distinctive feature is a ketosis which develops in the last week of gestation and progresses rapidly to a fatal termination.

The disorder is occasionally encountered in the general pregnant population of our rabbit colony, but it occurs with marked frequency in two groups of animals which show evidences of endocrine abnormality and transmit lethal mutations of that order. Moreover, in these groups the disease occasionally occurs with typical clinical and pathologic features in nonpregnant females.

The facts at hand indicate that the disease is a disorder of carbohydrate and fat metabolism, and that its association with pregnancy is due to the metabolic activity of the mother rather than to the presence of the products of conception. The increased susceptibility of the two abnormal groups is apparently a function of endocrine abnormality, and this susceptibility may be of such a degree that the typical disorder occurs in the absence of pregnancy.

FATTY CONDITION OF THE LIVER PRODUCED BY ALCOHOL AND A DIET RICH IN FAT. J. L. BOLLMAN and F. C. MANN, Mayo Clinic.

A diet rich in fat and poor in carbohydrate produces excessive fatness of the liver in dogs within from three to four weeks. No marked changes are produced before this period. If to the dogs receiving the fatty diet alcohol is administered by mouth (2 cc. for each kilogram of body weight twice daily) it produces only

mild symptoms of alcoholic intoxication, but within five days causes their livers to become twice as fat as those of animals on the same diet without alcohol. As this treatment is continued the fat increases rapidly so that within from ten to fifteen days the liver is more than 20 per cent fat. Other changes in the chemistry of the liver also occur; the amount of glycogen decreases to a low level, and the total amount of the phosphate compounds of the liver also decreases. No marked histologic changes can be demonstrated other than the infiltration by fat which seems similar to that which occurs after a longer time in dogs fed the same diet without alcohol. Several indications of impairment of the hepatic function may be obtained when the liver is fatty. The normal chemistry and the normal function return rapidly after the administration of carbohydrate to the animals.

THE PLASMA PROTEIN IN A DOG WITH AN ECK FISTULA. S. C. MADDEN (introduced by G. H. WHIPPLE), University of Rochester, N. Y.

Of three dogs with Eck fistulas, one showed unusual protein metabolism. On a diet in which protein was low but which was adequate for the maintenance of weight the concentration of protein in the plasma and the total amount of the circulating protein fell off toward a level critical for edema. Diets of high potency for the production of plasma protein in normal dogs showed low potency in this animal. On a diet in which protein was low but which allowed regeneration of both plasma protein and hemoglobin in a normal anemic animal, this dog, when anemic, showed ready regeneration of hemoglobin but an inability to maintain the plasma protein at a normal level.

THE EFFECT OF INTOXICATION AND INFECTION ON THE REGENERATION OF HEMOGLOBIN. F. S. ROBSCHEIT-ROBBINS, University of Rochester, N. Y.

A long-continued severe experimental anemia produced in dogs by withdrawal of blood offers an excellent opportunity for the study of the effect of various conditions on the formation of red cells and hemoglobin. Intoxication resulting from a sterile abscess or from endometritis has a marked influence on the regeneration of hemoglobin.

THE CHEMOTROPISM OF LEUKOCYTES IN RELATION TO THEIR RATE OF LOCOMOTION. M. MCCUTCHEON and H. M. DIXON (by invitation), University of Pennsylvania.

By chemotropism of leukocytes is understood a directional response on the part of the cells, which move toward a source of attraction (if the response is positive) but which move at random in the absence of an attracting body. Whether a leukocyte reacting chemotropically travels faster than one moving at random, that is, whether chemotropism has not only a directional but also a velocity component, appears not to have been decided. In order to answer this question, experiments were made in vitro with human polymorphonuclear leukocytes. Leukocytes were observed with the microscope, and with the help of a drawing ocular their paths were recorded on paper. From these records the rate of locomotion was computed (1) for leukocytes moving toward bacteria (or other attracting bodies) in the same microscopic field and (2) for leukocytes moving in parts of the same preparation so far distant from bacteria that their locomotion was at random. The sources of attraction used were Staphylococcus aureus, Staphylococcus albus and collodion particles (of about the size of bacteria). Leukocytes in the vicinity of each kind of particles were strongly attracted by them, but the rate of locomotion of the leukocytes was no greater than that of cells moving at random. For example, the mean rate of locomotion of leukocytes moving toward collodion particles was 35 microns per minute; for cells moving at random the rate was the same. It is concluded that the chemotropic response does not involve increase in the rate of locomotion.

THE MICROMETABOLISM OF LYMPHOCYTES: ITS RELATIONSHIP TO CONCEPTS OF BLOOD FORMATION. CARL V. MOORE (by invitation), B. K. WISEMAN and J. J. QUILLIGAN JR. (by invitation), Ohio State University.

What position the lymphocyte should occupy in theoretical concepts of blood formation continues to be one of the most important of the unanswered questions relating to the physiology and pathology of the blood-forming tissues. Hoping to supplement the evidence which tissue culture and morphologic studies have contributed, we have investigated the micrometabolism of these cells and have determined the bearing of the data on the problem. The technic has been made more applicable by the discovery of a rich source of lymphocytes which assays by supravital examination 96 to 99 per cent mature cells. These cells are obtained from the submucosal layer of the rabbit's appendix and are easily suspended in a fluid medium. The trauma and uncertainties which attend the slicing of tissues are thereby avoided, and optimal conditions for cell metabolism with individual units of known maturity and identity are attained. Measurements of the oxygen consumption and glycolytic activity of the cells have shown that normal mature lymphocytes, at least as found in the rabbit, have metabolic characteristics almost identical with those found to prevail in fixed connective tissue cells and not identical with those found in the primitive undifferentiated cells of embryonic mesenchyme. These studies indicate that normal lymphocytes are mature mesenchymal elements rather than primitive undifferentiated cells and are therefore probably not capable of transformation into other cells of the blood as is postulated by the unitarian theory of blood formation. A detailed presentation of the technic and the data will be given.

EFFECT OF TOTAL REMOVAL OF THE LIVER ON THE BLOOD FATS. STEPHEN MADDOCK, Boston City Hospital.

Studies were made of the fat of the blood plasma in dogs following hepatectomy. All the animals showed a consistent fall in the total lipids throughout the duration of the experiment. The drop in fats does not begin to be apparent until after the twelfth hour. These results are at variance from those previously reported, notably by Enderlen, Thannhauser and Jenks. Some additional information will be presented which tends to reconcile this discrepancy.

ARTERIAL HYPERTENSION: I. RESPONSE IN BLOOD PRESSURE AND BLOOD SUGAR TO INSULIN-INDUCED HYPOGLYCEMIA. M. PIJOAN (introduced by ELLIOTT C. CUTLER), Peter Bent Brigham Hospital, Boston.

The responses in blood pressure and blood sugar following intravenous administration of 20 units of insulin were studied in normal persons and in patients with arterial hypertension and with Addison's disease. In twenty-five normal persons the blood sugar fell to 40 mg. per hundred cubic centimeters; at this point there was a sharp rise in the blood pressure, averaging 40 mm. systolic and 10 mm. diastolic, and an elevation of 10 mg. of blood sugar also occurred. In twenty patients with essential hypertension the blood sugar fell to an average of 45 mg. per hundred cubic centimeters; at this point the blood pressure rose 56 mm. systolic and 22 mm. diastolic, and the blood sugar rose 20 mg. Four patients with Addison's disease had an initial drop in blood sugar to 15 mg. per hundred cubic centimeters, which was sustained for two hours, with a subsequent gradual rise, and no changes in blood pressure throughout the experiment.

Detailed studies were carried out not only on patients but on dogs. The dogs were subjected to the same treatment before and after adrenalectomy. The reaction to insulin previous to adrenalectomy was as in normal subjects, whereas subsequent to adrenalectomy there were no changes in blood pressure due to a critical hypoglycemia, which was sustained for several hours.

Conclusion.—The hypoglycemia induced by insulin calls forth a secretion of epinephrine, and this is responsible for the sudden hyperpiesis. The response of

hyperextensive patients to the epinephrine is excessive, whereas that of patients with Addison's disease is negligible.

CERTAIN HYDROXY-ALKYL-BENZENE ANTISEPTICS. F. W. HARTMAN and VICTOR SCHILLING (by invitation), Henry Ford Hospital, Detroit.

The studies cover investigations on di-hydroxy-di secondary hexylbenzene, di-hydroxyhexylethylbenzene, di-hydroxyhexylchlorobenzene, hexyl-p-chloro-m-cresol and related compounds, used alone and in combination with hydrochloric acid and other acids.

THE PROTHROMBIN CONTENT OF PLASMA IN VARIOUS ANIMAL SPECIES. K. M. BRINKHOUS (by invitation), E. D. WARNER (by invitation) and H. P. SMITH, University of Iowa.

The titration of the prothrombin in the plasma was carried out by a procedure recently devised. Special attention is given to the titers of a number of widely separated species. A comparison of these shows variations in the amount of prothrombin present and in the speed with which it can be converted into thrombin.

COAGULATION OF BLOOD BY PROTEOLYTIC ENZYMES. HARRY EAGLE (introduced by BALDUIN LUCKÉ) and TZVEE HARRIS (by invitation), University of Pennsylvania.

Citrated blood or plasma is coagulated by both trypsin and papain. The latter acts directly on the fibrinogen, converting it to an insoluble modification resembling fibrin. Trypsin, however, has no effect on fibrinogen but acts on prothrombin, converting it to thrombin. The amount of thrombin formed falls off sharply on either side of an optimal ratio of trypsin to prothrombin. This optimal ratio remains fairly constant over a wide range of concentrations, suggesting a stoechiometric relationship. Physiologic coagulation may involve a similar enzymic transformation of prothrombin to thrombin.

THE MECHANISM OF IMMUNITY IN TUBERCULOSIS: THE RÔLE OF CELLULAR AND EXTRACELLULAR FACTORS. MAX B. LURIE, Henry Phipps Institute, Philadelphia.

If a mixture of tubercle bacilli and melted agar is injected subcutaneously into normal rabbits the bacilli grow unhindered in the acellular agar islands at a great distance from the tissue cells. This they are able to do because the body fluids penetrate the agar. In rabbits vaccinated with B C G and in tuberculous rabbits the growth of the bacilli in such islands is markedly inhibited. The extent of growth may be determined by culture. The agar focus as a whole and the individual agar islands of the sensitized animal become surrounded by an extensive dense network of fibrin, while in the normal animal this barrier is less pronounced. Furthermore, both tubercle bacilli and particulate matter tend to be agglutinated in the acellular agar in the immunized animal, whereas in the normal animal both the bacilli and the carbon particles are largely dispersed. Again, in the normal animal the polymorphonuclears, which have no capacity to destroy tubercle bacilli, persist in the focus for a long time, and the mononuclears, with a slight capacity for destroying tubercle bacilli, are tardy in their appearance. In the vaccinated or tuberculous rabbit, on the other hand, the polymorphonuclears soon disappear from the site of reinfection, and their place is taken by rapidly mobilized mononuclear phagocytes, with a greatly enhanced capacity to destroy tubercle bacilli. Thus immunity to tuberculosis rests on factors which inhibit the growth of the bacilli of reinfection and which immobilize and destroy the organisms themselves.

All attempts to demonstrate in vitro the extracellular factor responsible for the inhibition of growth have met with no success.

The agar focus and the pleural exudate in response to the injection of dead tubercle bacilli in the vaccinated or the tuberculous rabbit are consistently more

acid than the corresponding agar focus and pleural exudate in the normal rabbit. The difference in p_H, however, is on the average only 0.2. The slightly more acid reaction of the agar focus of the vaccinated or the tuberculous animal is obviously insufficient to explain the marked inhibition of growth. Possibly the enzymes released into the agar by the mononuclears of the tuberculous or vaccinated rabbit explain the results. The more rapid mobilization of the mononuclears is apparently due to a nonspecific change in their reactivity or in the permeability of the blood vessels, for the same observation is made in the pleural exudate of tuberculous rabbits in response to the injection of aleuronat (an albuminoid substance plus lecithin) and starch. There is no consistent correlation between the p_H of an exudate and its leukocytic formula.

The agglutination of tubercle bacilli and of carbon particles in the tuberculous rabbit is apparently a property of the plasma.

ACTION OF PURIFIED ENZYMES ON CERTAIN VIRUSES. MALCOLM H. MERRILL (introduced by CARL TEN BROECK), Rockefeller Institute for Medical Research, Princeton, N. J.

The action of crystalline trypsin and chymotrypsin has been tested on four viruses. The virus of equine encephalomyelitis is inactivated by chymotrypsin but not by trypsin; the virus of pseudorabies is inactivated by both enzymes; the virus of swine influenza is not inactivated by either, and the virus of vaccinia resists the action of chymotrypsin but appears to be slowly inactivated by trypsin. The significance of these facts is discussed.

MORPHOLOGIC STUDY AND CLASSIFICATION OF THE RICKETTSIAE. HENRY PINKERTON and GEORGE M. HASS (by invitation), Harvard University.

Studies of the rickettsiae of typhus and spotted fever in tissue cultures (previously reported) have shown that each disease is represented by a characteristic pattern of cell infection. Rickettsia Prowazeki, the cause of typhus, distends the cytoplasm of its host cells without invading the nuclei. Dermacentroxenus Rickettsii, the cause of spotted fever, infects the cytoplasm of its host cells only sparsely but forms compact intranuclear clusters, often distending the involved nuclei. Important morphologic differences between the two organisms are also apparent when they are studied by this method. Attempts to classify the rickettsial diseases of all parts of the world on the basis of clinical criteria (in man and in experimental animals) have been unsuccessful largely because variations in the virulence of strains may render such criteria inconstant or even useless. Classification based on immunologic studies have been more successful but still leave much to be desired. The distinctive patterns of cell infection and the morphologic differences observed in tissue cultures have been used as a basis for a more accurate classification. The various strains studied have all given either the typhus picture or the spotted fever picture described. There has been no overlapping, and a single infected culture has always been sufficient for a definite diagnosis. Unidentified diseases have been identified readily by this method, and their identifications have later been confirmed by such other morphologic, immunologic and epidemiologic evidence as has become available.

Clinically various and geographically widely distributed diseases, such as Rocky Mountain spotted fever, Eastern (U. S.) spotted fever, Reimann's disease (caused by an atypical strain isolated in Minnesota) and *fièvre boutonneuse* (Mediterranean fever), have been found to be morphologically identical and to have identical patterns of cell infection. For purposes of classification, these constant morphologic and cytologic criteria are believed to be of much greater value than clinical criteria. The etiologic agents of these diseases are therefore regarded as strains or, at most, as varieties of Dermacentroxenus Rickettsii. Such clinical and minor immunologic differences as have been observed between louse-borne (human) and flea-borne (murine) typhus are likewise, in the absence of morphologic differences, regarded as subspecific. With the exception of the incompletely studied mite-borne

diseases, all reported human diseases that have been proved to be rickettsial may be classified, then, as typhus (etiologic agent, Rickettsia Prowazeki) and spotted fever (etiologic agent, Dermacentroxenus Rickettsii).

RELATION OF CERTAIN COCCOBACILLIFORM BODIES TO AN INFECTIOUS CORYZA OF FOWLS. JOHN B. NELSON, Rockefeller Institute for Medical Research, Princeton, N. J.

An infectious coryza of fowls, characterized by a slow onset of symptoms, the mean period of incubation being approximately two weeks, is considered. Exudate from the nasal tract of the infected bird is found to contain minute gram-negative coccobacilliform bodies, 0.5 micron or less in diameter, which are cultivable only in the membranes of fertile eggs or in tissue cultures. Such cultures are infective for normal birds, producing a coryza which in general resembles the naturally acquired disease. Evidence is presented that the coccobacilliform bodies are bacterial.

LYMPHOSARCOMA CELL LEUKEMIA. RAPHAEL ISAACS, Simpson Memorial Institute, University of Michigan.

Ten cases of lymphosarcoma terminating with a leukemoid blood picture have been studied. The condition was diagnosed early by biopsy. The predominant cell in the peripheral blood superficially resembles a lymphocyte but cytologically shows certain differentiating characteristics. The nucleus is oval or oblong, in some instances having one end thicker than the other, or is distinctly kidney-shaped or has one or more notches in the circumference. The sizes vary from 7.5 by 9 microns to 12 by 13.5 microns. The chromatin is coarsely reticular and somewhat spongy in structure, and there is quite a definite thickening of the nuclear wall, giving the appearance of a membrane that is part of the chromatin network. There is a single large nucleolus, eccentrically placed; rarely it is double. The cytoplasm is sparse and deeply basophilic; with brilliant cresyl blue it forms a fine blue lacework. The cells were identified by their similarity to lymphosarcoma cells (biopsy and necropsy material) which had been suspended in blood serum and later drawn out in films and stained with Wright's stain. The appearance of the leukemoid picture heralds a rapidly fatal outcome. This condition is considered as a true lymphosarcoma cell leukemia or a leukemoid stage of lymphosarcoma. It probably accounts for the description in the past of patients with lymphosarcoma terminating with lymphatic leukemia.

ASSOCIATION OF ANOXEMIA WITH EXPERIMENTAL HEPATIC LESIONS, PARTICULARLY THOSE INDUCED BY TOLUENDIAMINE. ALBERT M. SNELL, MILDRED ADAMS (by invitation) and JESSE L. BOLLMAN, Mayo Clinic.

Hepatic lesions produced by administering carbon tetrachloride and toluendiamine are associated with a decreased oxygen saturation of the hemoglobin of the arterial blood, the degree of unsaturation being roughly proportional to the acuteness and severity of the hepatic damage. The blood drawn from an animal that is in a state of severe intoxication from toluendiamine appears to be in an unstable state and shows reduction of oxyhemoglobin in vitro. In the presence of intoxication from toluendiamine there is marked anoxemia, which is at its height during the active phase and which disappears as the jaundice subsides and the oxygen capacity of the blood returns to normal. The evidence favoring the existence of a reducing agent in such specimens of blood is discussed.

Book Reviews

Pathologie und Klinik in Einzeldarstellungen. Edited by L. Aschoff, H. Elias, H. Eppinger, C. Sternberg and K. F. Wenckebach. **Band VII: Der endemische Kretinismus.** By Prof. Dr. F. de Quervain. Vorsteher der Chirurgischen Universitätsklinik Bern, and Prof. Dr. C. Wegelin, Direktor des Pathologisch-anatomischen Instituts der Universität Bern. Paper. Price, 24 marks. Pp. 206., with 120 illustrations. Berlin: Julius Springer, 1936.

This book is the product of the collaboration of a clinician and a pathologist and reflects the lifelong experience of the two best known authorities on Swiss goiter.

Cretinism is defined as a defective development of the body and mind, which is found throughout the world in regions where severe goiter is endemic. Thyroaplasia and acquired infantile myxedema, which occur also in regions free from goiter, are excluded, and it is stated that the term "sporadic cretinism" should be abolished. Familial dwarfism, chondrodystrophy and osteogenesis imperfecta must not be confused with cretinism.

In the chapter on the history of cretinism the fact is of interest that Paracelsus first recognized the relationship between endemic goiter and cretinism and that it was Theodor Kocher who attributed the symptoms of cretinism to insufficient thyroid function.

There are not yet reliable data on the frequency of cretinism in the different countries. Finkbeiner's statement that 1 per cent of the Swiss population is suffering from cretinism seems questionable, and Wagner-Jauregg's estimation that from 0.1 to 0.3 per cent of the population of Austria are cretins is regarded as too low. In Switzerland a slight decrease in the incidence of cretinism has been apparent during the last decade. Cretinism occurs in all parts of the world except in the polar regions and on the seacoast, while level regions where only mild goiter is endemic are almost completely free from cretinism. The Alps, the Pyrenees, the highlands of central Asia and the Andes of South America are the foremost centers of cretinism.

In the chapter dealing with the clinical manifestations of cretinism de Quervain discusses the body growth and the changes in the bones, skin, muscles, endocrine organs, intestinal tract, circulatory system and nervous system in persons with this condition. The psychic and inteliectual peculiarities of the cretin are competently described. There are three grades of cretinism. Cretins of the third grade are unable to talk, understand words or perform any work. Cretins in the second group are able to do certain forms of manual labor under constant supervision. Cretins belonging to the first grade are able to read and write, and they may learn some simple occupation; they are often married and have children. Many clinical photographs, roentgenograms and drawings are found in this chapter.

In the following sixty-three pages Wegelin gives a masterly account of the morbid anatomy in this disease. The thyroid changes are of chief importance. At birth the thyroid of the cretin is diffusely hyperplastic. The epithelial elements, however, undergo early degeneration, so that even in the first decade of life marked atrophy and sclerosis of the organ occur. In two thirds of the cases regenerative proliferation takes place and produces true adenomas. The large nodular goiters are of the parenchymatous type, and their functional value is low. Diffuse colloid goiters have never been observed in cretins. The thyroid tissue proper between the adenomas is atrophic, owing to pressure.

The anatomic changes in the other endocrine organs are regarded as secondary to the degeneration of the thyroid. The hypophysis is almost always enlarged. There is marked hyperplasia of the principal cells. The development of the gonads

is retarded, and the thymus gland shows early involution. The parathyroids, the pineal body and the adrenal glands show, as a rule, a normal structure.

Study of the brains of fourteen cretins revealed severe degenerative changes which apparently occurred early during fetal life. The disturbances in the development of bone are exhaustively described and illustrated. The low red cell count which is observed in many cretins is explained by the early transformation of the red bone marrow into fatty tissue. There are fifty-two excellent photographs and photomicrographs in this chapter.

The next chapter is devoted to the pathologic physiology of cretinism. The basal metabolism; the changes in the blood, which are cytologic as well as chemical and physical; the iodine content of the blood, and the structure of the capillaries of the skin are discussed. De Quervain concludes that all the available tests of function suggest deficient thyroid activity in the cretin, whether goiter is present or absent.

In the chapter on the pathogenesis both authors discuss the rôle of the thyroid and the questions of intermarriage, heredity and racial predisposition. The predominating factor in the etiology of cretinism is exogenic and is restricted to certain localities. The concluding chapter by de Quervain deals with the prophylaxis and treatment. As long as the essential cause of cretinism is not known, the prophylactic administration of iodine during pregnancy seems to be the only measure against this disease. The treatment of cretinism by thyroid medication is unsatisfactory.

The book contains an enormous amount of well arranged information. Its study is recommended to pathologists and clinicians in this country. Recent reports seem to indicate that cretinism is on the increase in North America and that in several generations this continent will be confronted by the same problem of cretinism as Switzerland faces today.

Cancer Commission Committee Studies of the California Medical Association. Price, 75 cents. Pp. 129. San Francisco: J. W. Stacey, Inc., 1936.

In 1931 the California Medical Association created a cancer commission. During the past five years the subcommittees of this commission have prepared and published in *California and Western Medicine* a series of studies and reports on the present status of the diagnosis and treatment of various forms of cancer. These articles constitute the text of this publication.

The manner of preparation, involving comprehensive and discriminating studies by two hundred physicians, surgeons, radiologists and pathologists, working in fourteen committees, marks the volume as unique.

The compilers will probably admit that the object of the volume, which is the education of physicians in the special field of the diagnosis and treatment of cancer, has been thoroughly accomplished in their own ranks.

The Committee on Radiology describes the apparatus required for roentgen and radium therapy and reviews the indications for radiation treatment of the major forms of malignant tumors. Their conclusions agree, in general, with those of the most progressive radiotherapeutists in this country and abroad. The conclusions are conservative and are notably and wisely influenced by the surgical point of view, but the report notes that in a substantial number of cases cure within five years by irradiation alone has been observed by the members of the committee in such organs as the stomach, brain, rectum, kidney, prostate and breast.

The report on radiology is so excellent in regard to the points covered as to suggest the advisability of enlarging its scope. Except in the case of uterine cancer, the exact dose is not discussed. The theory of the technic of divided doses, the importance of the size of the portal; the indications for unfiltered roentgen rays; the safeguards against telangiectasis of the skin, fibrosis of muscle and fragility of bones and teeth; the advisability of producing vesication of skin in certain cases, and many other theoretical and practical matters have been omitted, probably in the interests of brevity. A plain statement of the attitude of the com-

mittee on some of these points would probably be helpful to many radiologists. The supervoltage roentgen ray machine is not mentioned, although at least eight of these are now being used in this country.

The committees dealing with the various clinical departments of cancer follow the plan of presenting concisely but in complete detail all the essential data regarding the prevention, early diagnosis, treatment, complication, hazards, clinical methods of examination and even the surgical technic. In cases in which radiation is advised the details are left to the radiologist, and, as has been previously noted, are not presented in this report.

On points on which differences of opinion exist the opposing views are presented in a judicial manner. One of the outstanding features of the volume is the analysis of opinions of world authorities on the treatment of metastatic nodes of the neck in cancer of the oropharynx, by Otto H. Pflueger. This analysis shows that many surgeons still practice block dissection of the nodes, that there is a definite effort on the part of some but not all surgeons to distinguish between those cases in which metastasis is probable and those in which it is improbable, that preoperative irradiation is employed by the great majority and postoperative irradiation by many, that little attention is paid to the varying degrees of radiosensitivity of squamous and transitional cell tumors and of lympho-epithelioma and that few surgeons are familiar with the method of irradiating the nodes only when they are palpable and implanting radon seeds in those which resist this treatment. The theoretical principles on which the decision as to the treatment must be based are ably presented, but an account of the actual results as shown by cure within five years with the various methods is missing. It is, therefore, highly desirable that such results, where available, should be brought into the discussion.

Some positive statements in the report seem to call for reconsideration. Among these are the following: "For any 'malignancy' of the nasal passages, complete surgical removal is the ideal treatment" (page 51). "Tumors of peripheral nerve trunks are relatively uncommon, usually benign (though some are malignant), and are commonly amenable to surgical treatment" (page 86). "Treatment of retinal glioma. Immediate enucleation if growth is still confined to globe."

The uniform use of the term malignancy when the writer means cancer is a crude colloquialism. Malignancy is an abstract quality, while cancer is something concrete.

Taken as a whole, this report forms a compact, authoritative and extremely practical review of the diagnosis and treatment of cancer. The articles bear evidence of careful preparation and repeated revision. The book is a model for any other committees which may undertake such a task. The authors are to be congratulated on having made such a substantial contribution to the control of cancer.

Books Received

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